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Effects of microbial mitochondriotoxins from food and indoor air on mammalian cells

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Front cover (clockwise from top left corner): **1.** Hyphae of antimycin-producing *Streptomyces* sp. strain FS2 isolated from a grain of barley. **2.** Bacterial growth originating from grains of barley of tryptic soy agar. **3.** Gram stained cells of paenilide-producing *Paenibacillus tundrae* strain E8a. **4.** Boar spermatozoa dyed with the transmembrane potential-responsive dye JC-1.

“What I've started I must finish. I've gone too far to turn back.

Regardless of what may happen, I have to go forward.”

— Michael Ende, The Neverending Story

To Roni, for understanding.

And to my parents, for teaching me to always push a bit further.

Contents

List of original publications

This thesis is based on the following publications (listed in chronological order according to date of publication):

I. Andersson, M.A., Mikkola, R., **Rasimus, S.**, Hoornstra, D., Salin, P., Rahkila, R., Heikkinen, M., Mattila, S., Peltola, J., Kalso, S., Salkinoja-Salonen, M. 2010. Boar spermatozoa as a biosensor for detecting toxic substances in indoor dust and aerosols. Toxicology in Vitro 24(7): 2041-2052. doi:10.1016/j.tiv.2010.08.011.

II. **Rasimus, S.**, Mikkola, R., Andersson, M.A., Teplova, V.V., Venediktova, N., Ek-Kommonen, C., Salkinoja-Salonen, M. 2012. Psychrotolerant *Paenibacillus tundrae* Isolates from Barley Grains Produce New Cereulide-Like Depsipeptides (Paenilide and Homopaenilide) That Are Highly Toxic to Mammalian Cells. Applied and Environmental Microbiology 78(10): 3732-3743. doi:10.1128/AEM.00049-12.

III. **Rasimus-Sahari, S.**, Teplova, V.V., Andersson, M.A., Mikkola, R., Kankkunen, P., Matikainen, S., Gahmberg, C.G., Andersson, L.C., Salkinoja-Salonen, M. 2015. The peptide toxin amylosin of *Bacillus amyloliquefaciens* from moisture-damaged buildings is immunotoxic, induces potassium efflux from mammalian cells, and has antimicrobial activity. Applied and Environmental Microbiology 81(8): 2939-2949. doi:10.1128/AEM.03430-14.

IV. **Rasimus-Sahari, S.**, Mikkola, R., Andersson, M.A., Jestoi, M., Salkinoja-Salonen, M. 2016. *Streptomyces* strains producing mitochondriotoxic antimycin A found in cereal grains. International Journal of Food Microbiology 218: 78-85. doi:10.1016/j.ijfoodmicro.2015.11.007

The author’s contribution

I. Stiina Rasimus-Sahari conducted part of the experimental work related to the aerosol samples collected by electrofiltration, prepared control samples, and participated in writing the article.

II. Stiina Rasimus-Sahari planned and carried out the isolation and characterization of the strains (except for DNA fingerprinting), conducted the toxicity testing using the boar sperm bioassay, and participated in interpreting the results of all the experimental work. She wrote the majority of article and is the corresponding author.

III. Stiina Rasimus-Sahari prepared the bacterial extracts as well as planned, carried out and interpreted the results of the immunotoxicity tests with human macrophages and the boar sperm motility inhibition assay. She wrote the majority of article and is the corresponding author.

IV. Stiina Rasimus-Sahari planned and carried out the experimental work except for the HPLC-MS analyses and PK-15 cell experiments, interpreted the results, wrote the article, and is the corresponding author.

All coauthors have approved the above statements of contribution and consented to the publication of the papers as part of this thesis.

Abbreviations

$\Delta\Psi$	membrane potential
$\Delta\Psi_m$	mitochondrial membrane potential
$\Delta\Psi_p$	plasma membrane potential
BLM	black lipid membrane
BSMI assay	boar sperm motility inhibition assay
EC_{50} / EC_{100}	effective concentration 50% / 100% *
EHEC	enterohemorrhagic *Escherichia coli*
ELISA	enzyme-linked immunosorbent assay
HPLC-ESI-IT-MS	high-performance liquid chromatography with electrospray ionization ion trap mass spectrometry
HPLC-UV	high-performance liquid chromatography with ultraviolet detection
ICP-MS	inductively coupled plasma mass spectrometry
IL-18	interleukin 18
IL-1β	interleukin 1 beta
JC-1	5,5',6,6'-tetrachloro-1,1',3,3'-tetraethylbenzimidazolyl-carbocyanine iodide *
K_{OW}	octanol-water partition coefficient *
LC-MS	liquid chromatography-mass spectrometry
LPS	lipopolysaccharide
MeVal	N-methyl-L-valine
MIN-6	a murine pancreatic beta cell line
MNA	a murine neuroblastoma cell line
mtDNA	mitochondrial DNA
NLRP3	NOD-like receptor family, pyrin domain containing 3 *
O-Ala	lactic acid
O-Ile	2-hydroxy-3-methylpentanoic acid
O-Leu	2-hydroxisocaproic acid
O-Val	α-hydroxyisovaleric acid
PBMC	peripheral blood mononuclear cells *
PK-15	a porcine kidney tubular epithelial cell line
RLM	rat liver mitochondria
RP-HPLC	reversed-phase high-performance liquid chromatography

** see Glossary for more information*

Glossary

Amylosin	A heat-stable peptide toxin produced by *Bacillus* spp. which acts as a potassium and sodium ionophore (channel former).
Antimycin A	A toxin produced by species of *Streptomyces* which inhibits mitochondrial oxidative phosphorylation.
Bacterial toxin	A toxic compound produced by bacteria.
Cereulide	The heat-stable emetic toxin produced by *B. cereus* which acts as a potassium ionophore (carrier).
Cytokine	A signal molecule produced by cells of the immune system.
Effective concentration 50% / 100% (EC_{50} / EC_{100})	Toxin exposure concentration at which 50% or 100% of the exposed cells exhibited the measured effect.
Enniatin B	A mycotoxin produced by *Fusarium* spp. which acts as a cation ionophore (carrier).
HaCaT cells	A spontaneously immortalized human keratinocyte cell line originating from adult human skin and exhibiting normal differentiation.
Inflammasome	A cytoplasmic complex of molecules in cells of the innate immune system which triggers inflammatory responses by activating caspase-1, leading leads to active cytokine release.
Ionophore	A lipid-soluble compound that can transport ions across a lipid membrane. An ionophore can either act as a carrier or form a channel.
JC-1	A fluorogenic transmembrane potential-responsive dye which emits orange fluorescence when $\Delta\Psi > 140$ mV and green fluorescence when $\Delta\Psi \leq 100$ mV.
Macrophage	A white blood cell present in all tissues that removes foreign material by phagocytosis as part of the innate immune system. It can also activate adaptive immune reactions. Macrophages develop from monocytes.
Microbial toxin	A toxic compound produced by bacteria or fungi.
Mitochondriotoxin / mitochondrial toxin	A compound which adversely affects mitochondria or specific mitochondrial functions.

Moisture-damaged building	A building in which structures have been exposed to dampness leading to structural damage and microbial growth.
NLRP3	A widely studied inflammasome which activates caspase-1 and has a critical role in the human innate immune system.
Octanol-water partition coefficient (K_{OW})	A value depicting the polarity of a compound determined by comparing the solubility of the compound in octanol (non-polar) to the solubility in water (polar). The higher the value, the more non-polar the compound is. The value is often presented on a logarithmic scale (log K_{OW}).
Paenilide	A heat-stable peptide toxin produced by *Paenibacillus tundrae* which acts as a potassium ionophore (carrier).
Peptide toxin	A toxic compound consisting of amino acids linked together by peptide bonds.
Peripheral blood mononuclear cells	The fraction of blood cells containing lymphocytes, monocytes and dendritic cells.
Sick building syndrome	A term used to describe situations in which occupants of a building experience health effects that appear to be linked to time spent in the building but no specific illness or cause can be identified.
Stephacidin B	A dimeric alkaloid toxin produced by *Aspergillus* spp.
Valinomycin	A peptide toxin produced by *Streptomyces* spp. which acts as a potassium ionophore (carrier).

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Abstract

Humans are exposed to microbes and microbial compounds continuously via environmental exposure and food in addition to our own microbiome. This thesis reports new empirical findings concerning the types of mitochondrial toxins present in food and indoor air and the effects of these toxins on mammalian cells.

Dust and aerosolized compounds collected from moisture-damaged buildings were shown to have mitochondriotoxic effects detectable with a boar sperm motility inhibition (BSMI) assay. Toxin-producing bacteria isolated from the dusts and aerosols included producers of the mitochondrial toxins amylosin, cereulide, and valinomycin. In addition, aerosolized valinomycin and toxic chemical compounds emitted from building materials were detected using boar spermatozoa, demonstrating that the BMSI assay could be used to screen for mitochondrial toxicity in indoor air.

Amylosin, a mitochondriotoxin produced by *Bacillus* spp. from indoor air and food, was shown to be immunotoxic: exposure of human primary macrophages to nanomolar concentrations of amylosin stimulated the release of inflammatory cytokines interleukin 1β and interleukin 18. Amylosin is the first bacterial channel-forming ionophore toxin reported to have this effect. Low amounts of amylosin caused significant dose-dependent potassium ion efflux from human somatic cells and boar spermatozoa. Potassium efflux may be the trigger causing the observed cytokine release from macrophages after amylosin exposure. Amylosin also displayed antifungal and antibacterial activity which may give its producers a competitive advantage in mixed microbial communities.

Two previously unreported food safety hazards were identified from cereal grains. A novel heat-stable mitochondrial toxin named paenilide, similar to cereulide biochemically and in mode of action, was found to be produced by *Paenibacillus tundrae* isolated from barley grains. This finding shows that the genus *Paenibacillus* cannot be considered harmless for human health. Paenilide consisted of two components with molecular masses equal to those of cereulide and homocereulide but with longer chromatographic retention times, indicating more hydrophobic structures than those of the cereulide compounds. Paenilide acted as a lipophilic potassium ionophore, causing depolarization, swelling, uncoupling of oxidative phosphorylation, and loss of respiratory control in isolated rat liver mitochondria. Paenilide accelerated glucose consumption in porcine kidney epithelial cells, causing metabolic acidosis, and was cytotoxic to porcine and murine cells. Paenilide was as toxic as cereulide at nanomolar amounts, making it a possibly potent food poisoning

agent. Paenilide was produced at incubation temperatures down to +5°C, indicating potential toxin formation in chilled foods.

The second novel finding connected to cereal grains was the discovery of toxin-producing *Streptomyces* spp. in healthy-looking grains of barley and spring wheat. The barley grain isolates, belonging to the *Streptomyces albidoflavus* group, produced the mitochondriotoxic macrolide antimycin A, a known disrupter of oxidative phosphorylation. The cause of the mitochondrial toxicity of the spring wheat grain isolates, most closely related to *Streptomyces sedi*, remained unidentified. The toxicity of the isolates and grains was detected using the BSMI assay, which was sensitive enough to detect nanomolar contamination corresponding to 2 ng of antimycin A per barley grain. Antimycin A was more toxic towards porcine kidney epithelial cells than the mycotoxin enniatin B but less toxic than cereulide or paenilide. Exposure of porcine kidney epithelial cells to these four toxins accelerated glucose consumption and caused mitochondrial depolarization, indicating upregulation of glycolysis. Pancreatic insulin-producing beta cells, however, are not able to switch to glycolytic ATP production and undergo necrotic cell death upon exposure to mitochondrial toxins. Thus, consumption of grains contaminated with mitochondrial toxins may especially affect pancreatic functions.

In conlusion, the results of this thesis show that chronic exposure via indoor air or food to sub-lethal concentrations of mitochondriotoxins produced by spore-forming bacteria may be more common than known so far. This exposure may connect to the increasing worldwide incidence of western lifestyle diseases, such as diabetes, asthma, and allergies as well as cardiovascular and neurological disorders. The studied toxins are all heat-stable and produced by spore-formers able to withstand harsh environmental conditions, highlighting that preventing their presence and endurance in food and indoor environments is problematic.

Tiivistelmä (abstract in Finnish)

Ihmiset altistuvat jatkuvasti mikrobeille ja mikrobien tuottamille yhdisteille ympäristön ja ruoan sekä oman elimistön kautta. Tässä väitöskirjassa esitellään uutta kokeellisesti kerättyä tietoa elintarvikkeissa ja sisäilmassa esiintyvistä mitokondriomyrkyistä ja niiden vaikutuksia nisäkässoluihin.

Kosteusvaurioituneista rakennuksista kerättyillä pölyillä ja aerosoleilla osoitettiin olevan myrkyllisiä vaikutuksia mitokondrioita kohtaan. Myrkyllisyys havaittiin sian siittiöiden liikkuvuutta mittaavalla kokeella. Näytteistä eristettiin myrkyllisiä bakteereja, joiden joukossa oli kolmea tunnettua mitokondriomyrkkyä (amylosiini, kereulidi ja valinomysiini) tuottavia kantoja. Osoitettiin myös, että ilmassa aerosolina oleva valinomysiini sekä rakennusmateriaaleista haihtuvat mitokondriomyrkylliset kemikaalit voidaan havaita sian siittiöiden avulla. Sian siittiöiden liikkuvuutta mittaavaa koetta voitaisiin siis hyödyntää seulontamenetelmänä sisäilmanäytteiden tutkimisessa.

Sisäilmasta ja elintarvikkeista eristettyjen *Bacillus*-lajien tuottaman mitokondriomyrkyn, amylosiinin, todettiin vaikuttavan haitallisesti ihmisen immuunijärjestelmän soluihin: ihmisen primääristen makrofagien altistuminen nanomolaarisille amylosiinipitoisuuksille johti tulehdusreaktioita edistävien sytokiinien, interleukiini 1β:n ja interleukiini 18:n, vapautumiseen soluista. Amylosiini on ensimmäinen bakteeriperäinen kanavia muodostava ionoforinen myrkky, jolla tämä vaikutus on raportoitu. Alhaisten amylosiinimäärien osoitettiin aiheuttavan merkittävää annosriippuvaista kaliumionien vuotoa somaattisista ihmissoluista sekä sian siittiöistä. Kaliumvuoto saattaa olla tekijä, joka laukaisee sytokiinien vapautumisen amylosiinille altistuneista makrofageista. Amylosiinin havaittiin myös estävän sienten ja bakteerien kasvua, mikä voi tarjota amylosiinin tuottajille kilpailuedun monimuotoisissa mikrobiyhteisöissä.

Viljan jyvistä löydettiin kaksi aiemmin tunnistamatonta elintarviketurvallisuusvaaraa. Ohran jyvistä eristettiin *Paenibacillus tundrae* -lajin kantoja, jotka tuottivat uutta penilidi-nimistä kuumuutta kestävää myrkkyä, joka muistutti rakenteeltaan ja toiminnaltaan kereulidia. Tämä löytö osoittaa, että *Paenibacillus*-sukua ei voida pitää harmittomana ihmisterveyden kannalta. Penilidi koostui kahdesta yhdisteestä, joiden molekyylimassat vastasivat kereulidia ja homokereulidia, mutta joiden kromatografiset retentioajat olivat pidemmät viitaten hydrofobisempaan rakenteeseen kuin kereulidikomponenteilla. Penilidi toimi lipofiilisenä kaliumionoforina aiheuttaen depolarisaatiota, turpoamista, oksidatiivisen fosforylaation irtikytkeytymistä ja hengityksen hallinnan menetystä eristetyissä rotan

mitokondrioissa. Penilidi kiihdytti glukoosin kulutusta sian munuaisten epiteelisoluissa johtaen metaboliseen happamoitumiseen (asidoosi) ja oli sytotoksinen sian ja hiiren soluille. Penilidi oli yhtä myrkyllistä kuin kereulidi nanomolaarisilla pitoisuuksilla, joten penilidiä voidaan pitää mahdollisesti vahvana ruokamyrkytysten aiheuttajana. Penilidiä muodostui jopa +5 °C:een lämpötilassa, joten sitä voisi muodostua myös kylmäsäilytetyissä elintarvikkeissa.

Toinen viljan jyviin liittyvä uusi löydös oli myrkkyjä tuottavien *Streptomyces*-lajien löytyminen terveeltä näyttävistä ohran ja kevätvehnän jyvistä. Ohran jyvistä eristetyt bakteerikannat, jotka tunnistettiin *Streptomyces albidoflavus* -ryhmän jäseniksi, tuottivat mitokondrioille myrkyllistä makrolidiyhdistettä, antimysiini A:ta, joka tunnetaan oksidatiivisen fosforylaation häiritsijänä. Kevätvehnän jyvistä eristettyjen, *Streptomyces sedi* -lajille läheisimmin sukua olevien bakteerien myrkyllisyyden syy jäi tuntemattomaksi. Kaikkien eristettyjen bakteerikantojen sekä jyvien myrkyllisyys havaittiin sian siittiöiden liikkuvuutta mittaavalla kokeella, joka oli riittävän herkkä havaitsemaan saastumista, joka vastasi 2 ng antimysiini A:ta ohran jyvää kohden. Antimysiini A oli myrkyllisempi sian munuaisten epiteelisoluille kuin homemyrkky enniatiini B, mutta vähemmän myrkyllinen kuin kereulidi tai penilidi. Sian munuaisten epiteelisolujen altistuminen näille neljälle toksiinille kiihdytti glukoosin kulutusta ja aiheutti mitokondrioiden depolarisaation, mikä viittaa glykolyysin tehostumiseen. Sen sijaan haiman insuliinia erittävät betasolut, jotka eivät kykene glykolyyttiseen ATP-tuotantoon, ovat paljon haavoittuvaisempia ja päätyvät nekroottiseen solukuolemaan altistuessaan mitokondriomyrkyille. Mitokondriomyrkkyjen saastuttaman viljan syöminen voi siis haitata erityisesti haiman toimintaa.

Tämän väitöskirjan tulokset osoittavat, että krooninen altistuminen sisäilman tai ruoan kautta pienille määrille itiöivien bakteerien tuottamia mitokondriomyrkkyjä saattaa olla yleisempää kuin tähän saakka on oletettu. Tämän kaltainen altistuminen voi olla yhteydessä länsimaiseen elämäntapaan liittyvien tautien, kuten diabeteksen, astman, allergioiden, sydän- ja verisuonitautien sekä hermostollisten häiriöiden, maailmanlaajuisesti kohonneeseen esiintyvyyteen. Kaikki tässä työssä tutkitut myrkyt ovat kuumuutta kestäviä ja itiöivien, ankaria ympäristöoloja kestävien bakteerien tuottamia, joten niiden läsnäolon ja säilymisen estäminen elintarvikkeissa tai sisätiloissa on haastavaa.

1. Introduction and literature review

Humans have always been exposed to microbes everywhere from birth to death, through inhaled air and consumed food and water as well as all forms of surface contact, leading to the formation of our own internal microbiomes. Our bodies have adapted to life with bacteria and fungi, learning to live in harmony with some and combat others. However, over the past few decades, dietary transitions and the heightened level of hygiene especially in the Western world has diminished natural exposure to microbes and caused changes in our microbiomes as well as our reactions to external microbes. The buildings we live and work in have become more airtight and the air we breathe is filtered and controlled by mechanical ventilation, decreasing exposure to the natural microbiota present in untreated outdoor air and consequently increasing the exposure of inhabitants to any contaminants present in the indoor air. In addition, the food we eat is ever more processed to enhance the ease of use demanded by consumers and ensure the long shelf lives required by global trade, creating new types of risks to food safety.

It is hypothesized that the changes in how we interact with our environment have led to changes in the microbial load our bodies are exposed to, leaving our immune system with few actual enemies to defend against and thus causing a tendency to overreact. There is a growing awareness that the modern Western way of life is connected to the increased incidence of conditions caused by unnecessary immunological responses (asthma and allergy) (reviewed by Daley 2014, Julia et al. 2015) and inappropriate reactions to our own cells (autoimmune and autoinflammatory diseases such as type 1 diabetes, thyroid dysfunction, celiac disease, and inflammatory bowel disease) (reviewed by Egro 2013, Kondrashova et al. 2013, Serban 2015, Thorburn et al. 2014). On the other hand, we are most likely causing new types of exposures via our living environments and diet which our bodies have not yet become accustomed to, leading to unforeseen health impacts.

Against this background, there is an increasing need for information on what we are being exposed to and what the possible results of exposure are. To this end, discovering novel microbially produced toxins and understanding the response of mammalian cells to toxin-producing microbes is of great importance. There is also a need to expand the use of rapid methods suitable for preliminary investigation of the mammalian cell toxicity of microbially produced compounds. The present study aims at contributing in all these areas.

1.1. Microbes and microbial toxins in indoor air of moisture-damaged buildings

In modern societies, people spend the majority of their lives in indoor environments (IOM 2011). Indoor air quality is therefore considered an important factor affecting human health (Heseltine et al. 2009). Although exposure to microbes is essential for normal human health (e.g., the functioning of the digestive system) as well as the development and upkeep of the innate immune system (Jarchum and Pamer 2011), health risks related to microbial growth harbored in moisture-damaged buildings are of continuous and increasing concern. For example, indoor air bacteria have been shown to induce inflammatory and cytotoxic effects which could be related to ill-health symptoms of inhabitants in moisture-damages sites (Hirvonen et al. 2005).

Indoor dust and building materials contain nutrients in amounts sufficient for both fungal and bacterial growth, with the availability of water as the single most important growth limiting factor (Heseltine et al. 2009, Korpi et al. 1997). Excessive indoor moisture most likely therefore impacts microbial growth, and it is not surprising that moisture-damaged buildings are known as environments infested with diverse types of fungi and bacteria, many of which produce bioactive compounds (Heseltine et al. 2009, Thrasher and Crawley 2009, Täubel et al. 2011). Based on a review of published studies, it has been estimated that at least 20% of buildings in Europe, Canada, and the United States display signs of dampness problems (IOM 2004), and the prevalence of dampness or mold in homes in the USA may be up to 50% (Mudarri and Fisk 2007). Indoor dampness appears to also be an issue in developing countries, and overall the incidence of dampness in homes is more common in low-income communities in both developed and developing societies (Heseltine et al. 2009). It is considered likely that global climate change threatens to increase the amount of moisture-damaged buildings worldwide (Heseltine et al. 2009, IOM 2011).

People living and/or working in moisture-damaged environments experience a wide range of symptoms, sometimes referred to collectively as damp building related illness (Wolff 2011). Symptoms vary from headaches, tiredness, and respiratory tract problems, such as asthma, to chronic fatigue, pseudoallergic reactions, gastrointestinal and neurological problems, and acute inflammatory responses (Curtis et al. 2004, Portnoy et al. 2005, Putus 2014). It is worth noting that the term "sick building syndrome", occasionally used in the same context, actually refers to a wider group of building-related symptoms not solely due to moisture-related problems (Hodgson 1992, Wolff 2011).

Although no definite causal relationship between experienced symptoms and exposure to moisture damage has yet been shown, the prevalence of especially respiratory illness is reportedly higher among inhabitants of moisture-damaged buildings and evidence of an association between indoor dampness and asthma exacerbation, cough, wheeze, and upper respiratory tract symptoms has been found (Mendell et al. 2011). This is not surprising, since inhalation is considered the main exposure route leading to inflammatory, allergic, toxic or immunosuppressive effects through contact with epithelial cells and cells of the innate immune system within the lungs (Heseltine et al. 2009). An estimated 20% of asthma cases in the United States are connected to moisture-related issues of residential buildings (Mudarri and Fisk 2007), and a significant portion of occupational asthma in Finland is linked to moisture damage in the workplace (Piipari and Keskinen 2005). The ill-health effects of inhabiting damp buildings cause significant costs, making moisture-damaged buildings a risk for both public health and national economy (Husman 2002, Mudarri and Fisk 2007).

The most common fungal indicators of moisture-damaged sites are various species belonging to the genera *Aspergillus*, *Penicillium*, *Fusarium*, *Cladosporium*, *Chaetomium*, *Stachybotrys*, *Acremonium*, and *Trichoderma*; these genera include species known as toxin producers and inducers of allergic reactions (Heseltine et al. 2009, Putus 2014). Fungal mycotoxins, especially trichothecenes produced by *Stachybotrys chartarum*, appear to be more common in the indoor air and dust in damp buildings and are considered possible causative agents of ill-health symptoms in damp buildings, although evidence of an association is still lacking (Heseltine et al. 2009). Although fungal growth is present in most cases of moisture damage, it is now being realized that focusing solely on fungal growth does not necessarily reflect how “sick” a building is: bacteria as well as both bacterial and fungal toxins must be taken into account as well (Nevalainen et al. 2015, Täubel et al. 2011).

Bacteria related to moisture-damage have been less studied, but there is evidence that actinobacteria, especially streptomycetes and mycobacteria, are prevalent in damp indoor environments (Heseltine et al. 2009, Rintala et al. 2004). Toxins produced by *Streptomyces* spp. isolated from moisture-damaged buildings include valinomycin, nonactin, monactin, staurosporine, and chloramphenicol (Andersson et al. 1998b, Täubel et al. 2011). Cereulide-producing *Bacillus cereus* strains and amylosin-producing *Bacillus amyloliquefaciens* strains have also been isolated from the indoor environment of moisture-damage sites (Andersson et al. 2005, Mikkola et al. 2007). Other toxigenic bacteria isolated from moisture-damaged indoor spaces are *Bacillus simplex* and *Nocardiopsis* spp. (Peltola et al. 2001a, Peltola et al. 2001b). Although research suggests that indoor dust contains mainly Gram positive bacteria (Rintala et al. 2008), Gram negative bacteria appear more common in building materials (Verdier et al. 2014). It has been suggested that amounts of bacterial endotoxin (lipopolysaccharide, LPS) from Gram negative bacteria are higher in damp buildings, but contradictory reports also exist, making the role of endotoxin currently debatable (Heseltine et al. 2009).

1.2. Microbes and microbial toxins in food

Microbes affect food in many ways. While some microbes are beneficial and necessary for the production of certain foods (e.g. fermented foods, probiotics), many foods are also inviting growth environments for pathogenic bacteria and spoilage microbes. In the European Union, about 5 200 foodborne illness outbreaks involving 43 000 people, 6 000 hospitalizations and 11 deaths were reported in 2013 (EFSA and ECDC 2015), and it is estimated that 48 million cases of foodborne illness occur each year in the USA, leading to nearly 128 000 hospitalizations and 3 000 deaths (Scallan et al. 2011a, Scallan et al. 2011b). Food safety is of growing concern also in the developing countries of Asia, Africa, and South and Central America, where foodborne illness can be seen as a threat to both human health and overall economic growth (Akhtar et al. 2014). Worldwide, the incidence of diarrheal diseases in 2013 was reported to be 2.7 billion cases (Global Burden of Disease Study 2013 Collaborators 2015), and although this figure includes diarrheal diseases contracted via all kinds of routes in addition to food (e.g., contaminated water supplies and person-to-person contact), it highlights the widespread nature of these conditions. With the expansion of global trade and extended shelf lives of food products, controlling microbial growth in foods is thus becoming more and more crucial for ensuring the availability of safe food for the growing global population.

The control of food safety requires preventing the growth of food spoilage microbes and pathogens by physical, chemical, or biological methods. Food preservation via physical (e.g., high or low temperatures, reduction of water availability, ionizing radiation, high pressure) and chemical (e.g., addition of organic acids, enzymes, nitrites, sulfites, plant-derived compounds) methods was done already before the microbial causes of food safety issues were identified, whereas biopreservation (e.g., use of lactic acid bacteria and bacteriocins in non-fermented foods) is a more recent development in food technology (Doyle and Buchanan 2013).

The most relevant bacteria endangering food safety worldwide based on current knowledge are (in alphabetical order) *Bacillus cereus*, *Campylobacter* spp., *Clostridium botulinum*, *Clostridium perfringens*, enterohemorrhagic *Escherichia coli* (EHEC), *Listeria monocytogenes*, *Salmonella* spp., *Shigella* spp., *Staphylococcus* *aureus*, *Yersinia enterocolitica*, *Y. pseudotuberculosis*, and *Vibrio* spp. (Akhtar et al. 2014, Doyle and Buchanan 2013, EFSA and ECDC 2015, EVIRA 2015, Scallan et al. 2011b). In addition, *Cronobacter* spp. is considered an emerging foodborne pathogen (Doyle and Buchanan 2013). Table 1 presents the toxins produced by these bacteria which are linked to food poisoning symptoms. Of these toxins, cereulide produced by *B. cereus* is known as a mitochondrial toxin. In 2014, 16.1% of foodborne outbreaks in the European Union were attributed to bacterial toxins with an increase of 82% in the total amount of reported outbreaks caused by bacterial toxins compared to 2010 (EFSA and ECDC 2012, EFSA and ECDC 2015).

Table 1. Common bacterial toxins causing food poisoning.

Toxin	Producer	Toxic effect
Cytotoxic compounds		
Cytolethal distending toxin	*Campylobacter* spp.	Causes damage to DNA and disrupts mitosis leading to distension of host cells
CPE enterotoxin	*Clostridium perfringens*	Causes Ca^{2+} influx into host cells leading to cell death
Cytotoxin	*Salmonella* spp.	Inhibits host cell protein synthesis
Shiga toxin Stx1a	EHEC, *Shigella dysenteriae*	Inhibits host cell protein synthesis
Shiga toxin Stx2 variants	EHEC	Inhibits host cell protein synthesis
Cytotoxin K	*Bacillus cereus*	Causes host cell lysis by pore formation in cell membranes
Cytotoxic and hemolytic compounds		
Nhe enterotoxin	*Bacillus cereus*	Causes host cell lysis by pore formation in cell membranes
Hemolysin B	*Bacillus cereus*	Causes host cell osmotic lysis
Neurotoxic compounds		
Botulin	*Clostridium botulinum*	Inhibits signaling at nerve-muscle junctions preventing voluntary muscle contraction and leading to flaccid paralysis

Table 1. (cont.)

Toxin	Producer	Toxic effect
Diarrheagenic compounds		
Yst enterotoxin	*Yersinia. enterocolitica*	Disrupts fluid and electrolyte transfer in intestinal absorptive cells
SHET1 and SHET 2 enterotoxins	*Shigella* spp.	Causes secretion in the small intestine
Cholera enterotoxin Ctx	*Vibrio cholerae*	Increases Cl^- secretion and decreases NaCl absorption in intestinal cells leading to osmotic water flow into the intestinal lumen
Enterotoxin	*Salmonella* spp.	Causes fluid exsorption into intestinal lumen
Emetic compounds		
Cereulide	*Bacillus cereus*, *Bacillus weihenstephanensis*	Inhibits activity of mitochondria and induces vomiting by stimulating the afferent vagus nerve
Staphylococcal enterotoxins	*Staphylococcus aureus*	Induce vomiting by stimulating neural receptors in the abdomen and possibly act as superantigens causing overactivation of innate immunity

Compiled from chapters 10 – 21 of Doyle and Buchanan (2013).

Bacteria are naturally not the only harmful foodborne microbial agents. Mycotoxin production connected to fungal growth poses a serious threat for food safety of especially stored commodities such as grains. The mycotoxins most relevant for food and feed safety are aflatoxins produced by *Aspergillus* spp., ochratoxins produced by *Aspergillus* and *Penicillium* spp, and fumonisins, deoxynivalenol, and zearalenone, all produced by *Fusarium* spp. (Doyle and Buchanan 2013). Mycotoxins can have neurotoxic, nephrotoxic, hepatotoxic, carcinogenic, teratogenic as well as immuno-suppressive effects, and usually these effects are not immediately apparent but rather a result of long-term exposure (Doyle and Buchanan 2013). In recent years, beauvericin, enniatins, fusaproliferin, and moniliformin produced by *Fusarium* spp. have been noted as emerging mycotoxins (Jestoi 2008). Of these, enniatins and beauvericin have been shown to damage mitochondria (reviewed by Jestoi 2008). In addition to bacteria and fungi, pathogenic viruses and protozoan parasites can also be transmitted via food (Doyle and Buchanan 2013).

1.3. Mitochondria: powerhouses, protectors, and suicide initiators of mammalian cells

Mitochondria are organelles of eukaryotic cells. They are traditionally considered to consist of a smooth outer membrane and an inner membrane, highly folded into cristae, which together form two spaces, the intermembrane space and the matrix (Figure 1). The outer membrane is permeable to small molecules whereas the inner membrane is mainly impermeable and has a highly negative membrane potential referred to as the mitochondrial membrane potential, $\Delta\Psi_m$ (Scheffler 2008). Recent studies have shown that contrary to prior belief, mitochondria do not normally exist as static single organelles. Instead, although they can function in isolation, in cells they are mobile and form functional networks among themselves as well as interacting with other organelles such as the endoplasmic reticulum (Milane et al. 2015). In addition, mitochondria are the only organelles with their own genome of non-nucleic DNA, which is inherited maternally. This mitochondrial DNA (mtDNA) encodes 13 proteins which are essential for oxidative phosphorylation (Scheffler 2008).

Mitochondria play a crucial role in many metabolic pathways of eukaryotic cells. They are the site of several energy producing reactions (see section 1.3.1.) and they also participate in amino acid metabolism as well as the biosynthesis of heme, cardiolipin, ubiquinol (coenzyme Q), and iron-sulfur centers (Scheffler 2008). The importance of normal mitochondrial functions is highlighted by the phenomena linked to mitochondrial dysfunction: the growing field of mitochondrial medicine has identified numerous diseases connected to hereditary or sporadic mitochondrial mutations and the accumulation of mutations in mtDNA is considered a possible cause of aging and some age-related neurodegenerative diseases, such as Parkinson's and Alzheimer's diseases (Scheffler 2008). Mitochondria also participate in the regulation of the innate immune system (see section 1.3.2.) and autophagy (Galluzzi et al. 2012, Tait and Green 2012). In addition, mitochondria are known to control one of the two main pathways leading to apoptosis, also known as programmed cell death (Wojtczak and Zablocki 2008) (see section 1.3.3.), and they possibly take part in the cell signaling connected to necroptosis (a form of regulated necrosis) (Galluzzi et al. 2012).

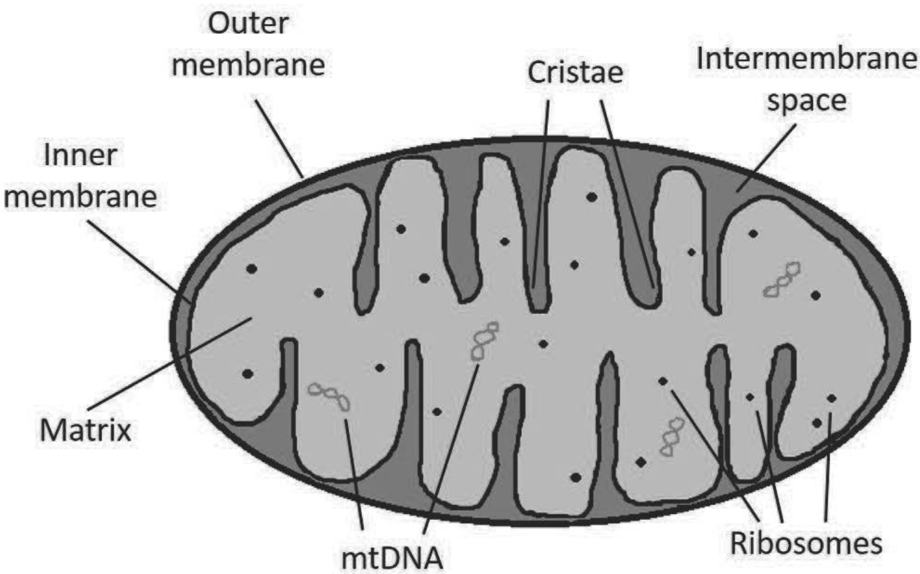

Figure 1. Schematic picture of the structure of a mitochondrion.

1.3.1. Energy production in mitochondria

Mitochondria are crucial as energy-producing organelles in mammalian cells, as oxidative phosphorylation, the major source of energy in the form of ATP for aerobic non-photosynthetic eukaryotic cells, only occurs inside mitochondria. Oxidative phosphorylation occurs in the folds (cristae) of the inner membrane where enzyme complexes I, II, III, IV, and V are located. Other metabolic processes involved in energy production, the Krebs cycle (citric acid cycle) and fatty acid oxidation, take place in the matrix. (Scheffler 2008)

The enzyme complexes participating in oxidative phosphorylation consist of multiple polypeptide subunits encoded by both mitochondrial and nuclear DNA. A schematic overview of oxidative phosphorylation is shown in Figure 2. In short, complexes I, II, III, and IV together form an electron transport chain that generates the proton motive force which complex V utilizes to produce ATP. NADH enters the respiratory chain via complex I, the NADH-ubiquinone oxidoreductase, which transfers electrons from NADH to ubiquinone, creating enough energy to transfer four protons from the matrix to the intermembrane space, thus affecting the proton gradient spanning the inner mitochondrial membrane. Complex II, the succinate-ubiquinone oxidoreductase, connects oxidative phosphorylation to the Krebs cycle: after oxidizing succinate to fumarate, complex II transfers the released electrons to ubiquinone. Complex III, the ubiquinol-cytochrome-c reductase or bc_1 complex, then transfers electrons from the reduced ubiquinone to cytochrome *c* in two steps while simultaneously translocating four protons from the matrix to the intermembrane space. Complex IV, the cytochrome-c oxidase, catalyzes the transfer of electrons from cytochrome *c* to the terminal electron acceptor, oxygen, producing primarily water but also reactive oxygen species (ROS); this occurs yet again in conjunction with the transfer of four protons into the transmembrane space. Finally, complex V, the F_1F_0-ATPase or ATP synthase, utilizes the proton motive force, generated by the proton gradient, as an energy source to drive the release of ATP synthesized within the complex using ADP and inorganic phosphate, thus turning kinetic energy into biochemical transferable energy. It is estimated that the production of one ATP requires the translocation of 3 or 4 protons back down the proton gradient into the mitochondrial matrix. (Nijtmans et al. 2004, Scheffler 2008)

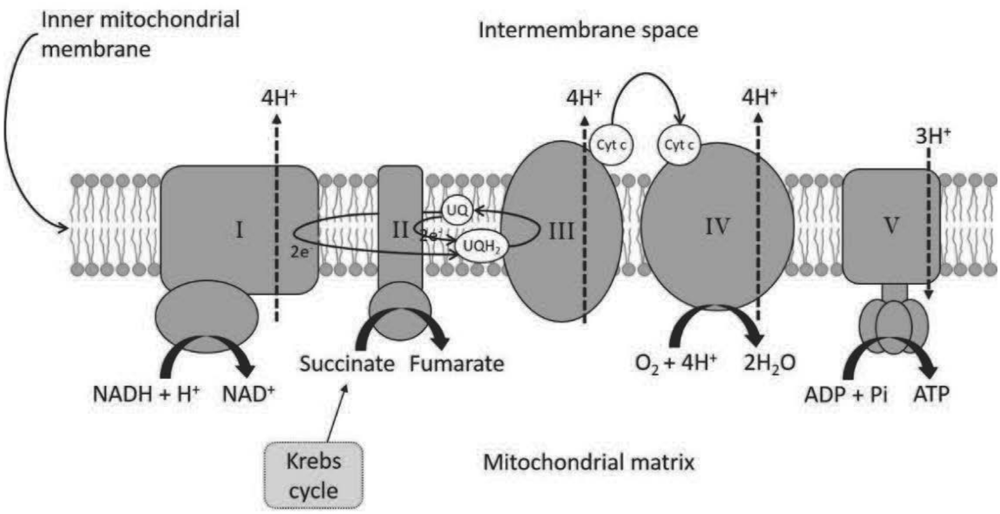

Figure 2. Schematic representation of the steps and complexes involved in oxidative phosphorylation.
I: NADH-ubiquinone oxidoreductase; II: succinate-ubiquinone oxidoreductase; III: ubiquinol-cytochrome-c reductase (bc_1 complex); IV: cytochrome-c oxidase; V: F_1F_0-ATPase (ATP synthase); NADH: reduced nicotinamide adenine dinucleotide; NAD^+: oxidized nicotinamide adenine dinucleotide UQ: ubiquinone; Cyt c: cytochrome *c*; ADP: adenosine diphosphate; Pi: inorganic phosphate; ATP: adenosine triphosphate.

1.3.2. Mitochondria and innate immunity

Mitochondria participate in different functions of the human innate immune system. They appear to play a role in regulating antiviral immunity and lyzosome-dependent degradation of unwanted cytosolic components (autophagy), and the reactive oxygen species produced during oxidative phosphorylation act as innate immunity signaling molecules (reviewed by Tait and Green 2012). Recent research indicates that mitochondria participate in the activation of the NLRP3 inflammasome (also known as NALP3 and cryopyrin), a cytosolic protein complex responsible for triggering inflammatory responses by activating caspase-1 which leads to the release of active cytokines IL-1β and Il-18 (Elliott and Sutterwala 2015, Sutterwala et al. 2014).

The NLRP3 inflammasome has been studied extensively since it was first described by Martinon et al. in 2002. It has a crucial role in activating inflammatory cytokine production and seems to be the most relevant inflammasome from a clinical point of view, as it can be activated by a wide range of diverse stimuli (reviewed by Abderrazak et al. 2015) and is implicated in the development of many significant non-infectious diseases, such as type 2 diabetes, obesity, inflammatory bowel disease, atherosclerosis, gout, Alzheimer's disease, as well as acute and chronic respiratory diseases (reviewed by De Nardo et al. 2014, Hosseinian et al. 2015, Leemans et al. 2011, Novak and Mollen 2015). Although mitochondrial dysfunction is not a mandatory requisite for NLRP3 activation (Muñoz-Planillo et al. 2013), it has been put forward as a key activator of the NLRP3 inflammasome, as numerous signals produced by damaged mitochondria (e.g., release of reactive oxygen species or mtDNA and Ca^{2+} efflux into the cytoplasm) have been shown to stimulate inflammasome assembly (reviewed by Gurung et al. 2015).

Activation of the NLRP3 inflammasome requires two signals. First, a priming signal upregulates the synthesis of NLRP3 and its post-translational modification, which is necessary for its oligomerization into an active form. The second step, which leads to the formation of an active NLRP3 inflammasome, can be triggered by many different types of stimuli, generally called danger signals, such as extracellular ATP, pore-forming toxins, phagocytized crystalline molecules (e.g., asbestos, silica), and endoplasmic reticulum stress. It is worth noting that although diverse stimuli can activate NLRP3, potassium efflux appears to always be required. Mitochondrial dysfunction is also considered an activator of the NLRP3 inflammasome, indicating that NLRP3 can sense mitochondrial damage and could possibly act as a monitor of mitochondrial integrity in cells. Moreover, it has been suggested that mitochondria act as a scaffold for NLRP3 inflammasome assembly in the activation process. (Elliott and Sutterwala 2015, Sutterwala et al. 2014)

1.3.3. Mitochondria and apoptosis

Apoptosis (programmed cell death) can occur via two pathways, the extrinsic death receptor pathway and the intrinsic mitochondrial pathway. The first step in the mitochondrial pathway is the controlled loss of the highly negative (−160 to −180 mV) mitochondrial inner membrane potential (loss of $\Delta\Psi_m$), which leads to the termination of ATP production and the release of mitochondrial proteins, including cytochrome *c*, into the cytosol, triggering a catalytic process which ends in cell death (Galluzzi et al. 2012, Milane et al. 2015, Scheffler 2008). The controlled loss of $\Delta\Psi_m$ can occur as a result of pore formation in the outer mitochondrial membrane by pro-apoptotic proteins of the Bcl-2 family, activated due to cellular stress such as DNA damage, UV radiation, Ca^{2+} influx or oxidative stress, or following a phenomenon termed mitochondrial permeability transition, in which the inner mitochondrial membrane becomes permeant to small solutes (Galluzzi et al. 2012, Milane et al. 2015).

1.4. Mitochondrial toxins

Since mitochondria are crucial for mammalian cells in many ways, mitochondrial dysfunction has serious consequences. Mitochondrial toxicity induced by pharmaceutical drugs has led to market withdrawals, making it a focus of growing interest for the pharmaceutical industry (Dykens and Will 2007). There is also rising concern about the mitochondrial toxicity of environmental toxins such as the widely used antibacterial agent triclosan (Ajao et al. 2015, Newton et al. 2005), the herbicide paraquat, the piscicide and pesticide rotenone, cyanide, heavy metals and cigarette smoke (reviewed by Meyer et al. 2013). On the other hand, the crucial role of mitochondria in controlling cell death makes mitochondriotoxic substances interesting as possible agents for anticancer therapies (Fantin and Leder 2006), although thus far none of these “mitocans” have successfully passed clinical trials (reviewed by Modica-Napolitano and Weissig 2015).

Examples of microbially produced mitochondrial toxins are listed in Table 2. Toxins which act as ionophores cause depolarization of the mitochondrial membrane (loss of $\Delta\Psi_m$), which affects mitochondrial functioning in many ways (Wallace and Starkov 2000). The production of ATP ceases as oxidative phosphorylation becomes uncoupled (no proton motive force) and the influx of ions causes influx of water and swelling of the mitochondria. Changes also occur in the mitochondrial matrix and mtDNA (Coppey et al. 1999), and ultimately depolarization leads to mitophagy (autophagy of mitochondria) (Lemasters 2014) or cell death by apoptosis as described above (section 1.3.3). Other toxins inhibit the action of specific proteins relevant for mitochondrial functions, such as myxothiazol which inhibits the second step in the transfer of electrons to cytochrome *c* within complex III of the mitochondrial electron transport chain (described in section 1.3.1) (Scheffler 2008).

The following sections present the mitochondrial toxins studied in this thesis work. The known chemical structures of antimycin A, cereulide, enniatin B, and valinomycin are presented in Figure 3.

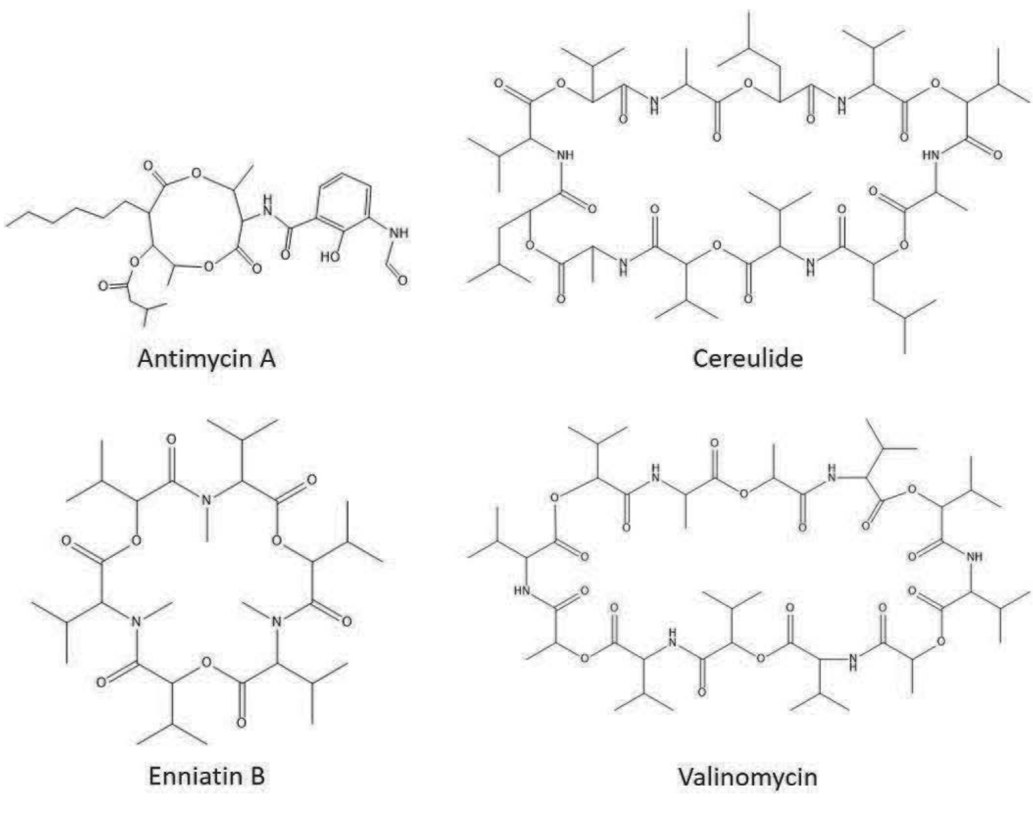

Figure 3. Structures of antimycin A, cereulide, enniatin B, and valinomycin.

Table 2. Examples of toxic microbial compounds affecting mammalian cell mitochondria.

Compound	Producer	References
Inhibitors of oxidative phosphorylation complex I		
Aurachin A and B	*Stigmatella aurantiaca*	Friedrich et al. 1994
Kalkitoxin	*Moorea producens*	Morgan et al. 2015
Myxalamides A-D	*Myxococcus xanthus*, *Stigmatella aurantiaca*	Steglich et al. 2000
Phenalamides A-C	*Myxococcus stipitatus*	Steglich et al. 2000
Phenoxan	*Polyangium* spp.	Friedrich et al. 1994
Piericidin A	*Streptomyces mobaraensis*	Friedrich et al. 1994
Thiangazole	*Polyangium* spp.	Friedrich et al. 1994
Inhibitors of oxidative phosphorylation complex II		
3-Nitropropionic acid (3-NPA)	*Arthrinium* sp., *Aspergillus flavus*, *Aspergillus oryzae*, *Penicillium atrovenetum*	Francis et al. 2013
Inhibitors of oxidative phosphorylation complex III		
Antimycin A	*Streptomyces* spp.	Steglich et al. 2000
Funiculosin	*Penicillium funiculosum Thom*	Nelson et al. 1977
Myxothiazol	*Myxococcus fulvus*	Thierbach and Reichenbach 1981
Stigmatellin	*Stigmatella aurantiaca*	Kunze et al. 1984
Inhibitors of oxidative phosphorylation complex IV		
Hydrogen cyanide	*Chromobacterium violaceum*, *Pseudomonas aeruginosa*, *Pseudomonas fluorescens*, *Anacystis nidulans*, *Nostoc muscorum*, *Plectonema boryanum*, *Rhizobium leguminosarum*	Blumer and Haas 2000
Metarhizin A	*Metarhizium flavoviride*	Katou et al. 2014
Inhibitors of oxidative phosphorylation complex V		
Aurovertins A and D	*Calcarisporium arbuscula*	Linnett and Beechey 1979
Citreoviridin	*Penicillium* spp.	Linnett and Beechey 1979
Efrapeptin	*Tolypocladium* spp.	Cross and Kohlbrenner 1978, Krasnoff and Gupta 1991
Oligomycin A, B, and C	*Streptomyces diastatochromogenes*	Linnett and Beechey 1979
Calcium ionophores		
Calcimycin	*Streptomyces chartreusensis*	Reed and Lardy 1972
Ionomycin	*Streptomyces conglobatus*	Liu and Hermann 1978
Listeriolysin O	*Listeria monocytogenes*	Stavru and Cossart 2011
Sodium ionophores		
Monensin	*Streptomyces cinnamonensis*	Steglich et al. 2000

Table 2. (cont.)

Compound	**Producer**	**References**
Potassium ionophores		
Amylosin	*Bacillus amyloliquefaciens*, *Bacillus subtilis*, *Bacillus mojavensis*	Apetroaie-Constantin et al. 2009, Mikkola et al. 2004, Mikkola et al. 2007
Bafilomycin	*Streptomyces griseus*	Teplova et al. 2007
Beauvericin	*Fusarium* spp.	Tonshin et al. 2010
Cereulide	*Bacillus cereus*	Mikkola et al. 1999
Enniatins A, A1, B, and B1	*Fusarium* spp.	Tonshin et al. 2010
Narasin	*Streptomyces aureofaciens*	Wong et al. 1977
Nonactin	*Streptomyces* spp.	Steglich et al. 2000
Salinomycin	*Streptomyces albus*	Mitani et al. 1975
Valinomycin	*Streptomyces* spp.	Mathews et al. 2000
Inducers of the mitochondrial apoptosis pathway		
α-toxin (α-hemolysin)	*Staphylococcus aureus*	Rudel et al. 2010
EspF and Map	EHEC	Rudel et al. 2010
Lethal toxin	*Clostridium sordellii*	Rudel et al. 2010
Microcystins	*Anabaena* spp., *Microcystis* spp., *Nostoc* spp., *Planktothrix* spp., *Aphanizomenon* spp.	Campos and Vasconcelos 2010
Omp38	*Acinetobacter baumanii*	Rudel et al. 2010
Panton-Valentine leukocidin	*Staphylococcus aureus*	Rudel et al. 2010
Pneumolysin	*Streptococcus pneumoniae*	Rudel et al. 2010
PorB porin	*Neisseria gonorrhoeae*	Rudel et al. 2010
Shiga toxin 1	*Escherichia coli*	Lee et al. 2005
Toxin A, toxin B (TcdA, TcdB)	*Clostridium difficile*	Rudel et al. 2010
VacA toxin subunit p34	*Helicobacter pylori*	Rudel et al. 2010
Other		
Diphteria toxin subunit A: inhibits energy production (exact mechanism unknown)	*Corynebacterium diphtheriae*	Abraham et al. 1982
Endotoxin / Lipopolysachharide (LPS): inhibits energy production (exact mechanism unknown)	Gram-negative bacteria	Jeger et al. 2015
Gramicidins: form cation-specific pores	*Brevibacillus brevis*	Mathews et al. 2000
Nigericin: K^+/H^+ antiport	*Streptomyces* spp.	Mathews et al. 2000, Steglich et al. 2000
Staurosporine: inhibits protein kinase C (affects complex V)	*Streptomyces staurosporeus*	Gani and Engh 2010

1.4.1. Amylosin

Amylosin is a heat-stable, acid and base resistant peptide toxin (1,197 Da) produced by strains of *Bacillus amyloliquefaciens*, *B. subtilis* and *B. mojavensis* (Apetroaie-Constantin et al. 2009, Mikkola 2006, Mikkola et al. 2007). It was first identified from fungicidic *B. amyloliquefaciens* strains isolated from moisture-damaged indoor spaces (Mikkola et al. 2004). It is moderately lipophilic with a log K_{OW} between 3 and 4 (Mikkola 2006). The structure of amylosin has not been elucidated, but it is a linear or cyclic peptide known to contain a polyene chromophore and the amino acids tyrosine, proline, leucine / isoleucine, aspartic acid / asparagine, and glutamic acid / glutamine (Mikkola et al. 2007).

Amylosin forms cation-selective channels in lipid membranes with high affinity for potassium ions compared to sodium or calcium ions (Mikkola et al. 2004, Mikkola et al. 2007). It inhibits boar sperm motility, is cytotoxic to feline lung cells and human neural cells, depolarized mitochondria in mammalian cells, and causes uncoupling of oxidative phosphorylation and swelling in isolated mitochondria at nanomolar concentrations in the presence of potassium and sodium ions (Apetroaie-Constantin et al. 2009, Mikkola et al. 2004, Mikkola et al. 2007).

1.4.2. Antimycin A

Antimycin A, a mixture of four structurally similar compounds (A_1 – A_4; 507 to 549 Da) (Steglich et al. 2000), belongs to the macrolide antibiotics, although it currently has no therapeutic use due to its toxicity. First isolated in 1947 by Leben & Keitt as a compound active against phytopathogenic fungi (as reported by Riclea et al. 2012, Steglich et al. 2000), antimycin A is produced by several species of *Streptomyces*, including *S. antibioticus*, *S. albus*, *S. albidoflavus* and *S. turgidiscabies* (Kotiaho et al. 2008, Olano et al. 2014, Seipke et al. 2011, Vezina et al. 1976, Yan et al. 2010). The antimycin A compounds contain a bislactone ring where threonine is linked by an amide bond to 3-N-formylsalicylic acid, with compounds A_1-A_4 having different alkyl chains at C-7 (Steglich et al. 2000). Antimycin A is lipophilic with a log K_{OW} of 5.71 (Gulden et al. 2001).

Antimycin A inhibits mitochondrial oxidative phosphorylation by interfering with electron transport within complex III (Figure 2), specifically preventing electron transfer from cytochrome *b* to cytochrome c_1 (Mathews et al. 2000, Scheffler 2008). It is toxic to birds, mammals and fish but not plant cells, as plant cell mitochondria possess an alternative oxidase which enables them to bypass complex III (Lennon et al. 1971, Millar et al. 2011). Antimycin A causes apoptosis in human liver cells as well as bovine and human vascular endothelial cells and induces cell death in osteoblasts, and it has been suggested that these reactions may lead to atherosclerosis, hypertension, diabetes, and osteoporosis (Chen and Yan 2005, Choi and Lee 2011, Sena et al. 2013, You and Park 2010). Antimycin A is of interest as a cancer therapy agent model due to its ability to reverse multidrug resistance in certain types of tumor cells (Tzung et al. 2001).

1.4.3. Cereulide

Cereulide is a non-ribosomally produced cyclic dodecadepsipeptide toxin (1,152 Da) produced by emetic strains of *Bacillus cereus* and *B. weihenstephanensis* (reviewed by Stenfors Arnesen et al. 2008). It was first identified by Agata et al. from a strain of *B. cereus* isolated from an individual with emetic food poisoning (Agata et al. 1994). Cereulide is lipophilic (calculated log K_{OW} = 5.96 to 7.46) (Mikkola et al. 1999, Teplova et al. 2006) and heat-stable up to 150 °C (Rajkovic et al. 2008). The amino acid structure of cereulide is cyclo(D-*O*-Leu-D-Ala-L-*O*-Val-L-Val-)$_3$ (Agata et al. 1994). Recently, it has been reported that there are at least 19 structural cereulide variants which display varying cytotoxicity (Marxen et al. 2015a).

Cereulide is an ionophore which forms stable complexes with monovalent cations, functioning as a carrier with high selectivity for potassium ions compared to sodium, calcium or ammonium ions (Mikkola et al. 1999, Teplova et al. 2006). It inhibits boar sperm motility, is cytotoxic to several human cell lines and porcine pancreatic beta cells, causes loss of $\Delta\Psi_m$ and osmotic swelling of mitochondria due to the uptake of potassium ions, and induces emesis in mammals (Agata et al. 1995, Andersson et al. 1998a, Andersson et al. 2007, Jääskeläinen et al. 2003, Mikkola et al. 1999, Virtanen et al. 2008). Cereulide inhibits sperm motility by causing potassium efflux from the cytoplasm, leading to hyperpolarization of the plasma membrane, and also causes potassium influx into mitochondria, causing loss of $\Delta\Psi_\mathrm{m}$ (Andersson and Salkinoja-Salonen 2005). The toxic dose causing emesis in humans is estimated as ≤8 μg kg^{-1} body weight (Jääskeläinen et al. 2003). Fatal and near-fatal cases of food-related cereulide intoxications have been connected to liver failure and metabolic acidosis caused by damage to mitochondria (Dierick et al. 2005, Ichikawa et al. 2010, Mahler et al. 1997, Naranjo et al. 2011, Shiota et al. 2010). Sub-emetic concentrations of cereulide (<1 ng ml^{-1}) have been shown to adversely effect intestinal epithelial cells (Rajkovic et al. 2014). The resistance of cereulide to heat, acids and proteolysis enhances its ability to maintain structural integrity in cooked foods and the digestive tract, increasing the risk of foodborne intoxication (Stenfors Arnesen et al. 2008).

1.4.4. Enniatin B

Enniatin B is a lipophilic (log K_{OW} = 6.9) cyclic hexadepsipeptide mycotoxin (640 Da) produced by *Fusarium* spp. and *Verticillium hemipterigenum* (reviewed by Sy-Cordero et al. 2012). It belongs to a family of closely related compounds of which enniatin A was first isolated by Gäumann et al. in 1947 from the mycelium of *Fusarium orthoceras* var. *enniatinum* as a compound with *in vitro* bioactivity against mycobacteria (Gäumann et al. 1947). The amino acid structure of enniatin B is cyclo(D-*O*-Val-L-MeVal-)$_3$ (Blais et al. 1992).

Enniatin B acts as a channel-forming cation ionophore with high selectivity for potassium ions (Kamyar et al. 2004, Tonshin et al. 2010). It inhibits boar sperm motility, is cytotoxic to murine macrophages, porcine kidney cells, heart muscle cells of guinea pigs, human liver carcinoma cells as well as fetal lung fibroblast-like cells, and causes loss of $\Delta\Psi_m$ and swelling of mitochondria (Hoornstra et al. 2003, Jestoi 2008, Sy-Cordero et al. 2012, Tonshin et al. 2010). Enniatin B has also been shown to have fungicidic activity (reviewed by Sy-Cordero et al. 2012). Enniatin B is considered an emerging mycotoxin and has only recently been identified as a possible hazard for food and feed safety (Jestoi 2008).

1.4.5. Valinomycin

Valinomycin is a cyclic dodecadepsipeptide toxin (1,111 Da) which acts as a lipophilic (calculated log K_{OW} = 4.49 to 5.99) potassium carrier similarly as cereulide (Mikkola et al. 1999, Teplova et al. 2006). Valinomycin was first isolated in 1955 by Brockmann and Schmidt-Kastner as an antibiotic effective against *Mycobacterium tuberculosis* produced by a strain of *Streptomyces fulvissimus*, and since then producers have been found to belong to several species, including *S. griseus*, *S. tsusimaensis*, *S. anulatus*, *S. exfoliatus* and *S. araujoniae* (Andersson et al. 1998b, Brockmann and Schmidt-Kastner 1955, Matter et al. 2009, Silva et al. 2014). The amino acid structure of valinomycin is cyclo(L-Val-D-*O*-Val-D-Val-L-*O*-Ala-)$_3$ (Steglich et al. 2000).

Valinomycin is a highly toxic compound which uncouples oxidative phosphorylation, inhibits boar sperm motility, and causes loss of $\Delta\Psi_m$ and osmotic swelling of mitochondria in the presence of potassium ions (Andersson et al. 1998b, Hoornstra et al. 2003, Steglich et al. 2000). It has also been shown to affect human natural killer cells, causing inhibition of cytokine production and apoptosis (Paananen et al. 2000). Although valinomycin is very similar to cereulide in many ways, it has a lower affinity for potassium ions than cereulide, making it less toxic than cereulide to mammalian cells at physiological potassium concentrations (Teplova et al. 2006).

1.5. Effects of exposure to mitochondrial toxins

1.5.1. Acute effects

Structural differences in the respiratory chain enzymes of plants, animals and fungi lead to differences in sensitivity towards toxins affecting mitochondrial respiration. In mammals, toxins which inhibit or uncouple oxidative phosphorylation accelerate the use of oxygen in conjunction with decreased production of ATP. This results in cytotoxic hypoxia, leading to hyperpnea (increased depth of breathing) and metabolic acidosis as a result of glycolytic ATP production and lactate accumulation (Wallace and Starkov 2000). Observable symptoms include muscle weakness, fatigue, headaches, nausea, and confusion as well as cardiovascular symptoms such as hypotension and aggravated myocardial angina (Wallace and Starkov 2000). Acidosis provoked by aerobic glycolysis combined with mitochondrial depolarization after exposure to the ionophoric mitochondriotoxin triclosan has been shown to cause necrotic cell death (Ajao et al. 2015). In addition to the inhibitory effects, mitochondrial toxins affecting complex III, such as antimycin A, cause increased generation of harmful reactive oxygen species (Wallace and Starkov 2000).

Several mycotoxins presented in Table 2, such as enniatin B, citreoviridin and 3-NPA, occur naturally in food crops like grains, rice and sugar cane (Almeida et al. 2012, Francis et al. 2013, Jestoi et al. 2004a, Jestoi et al. 2004b). 3-NPA as well as the bacterial toxins cereulide and amylosin are known to cause symptoms of food poisoning following acute exposure, with cereulide and 3-NPA connected to fatal human cases (Apetroaie-Constantin et al. 2009, Francis et al. 2013, Stenfors Arnesen et al. 2008). Citreoviridin has been connected to cases of thiamin (vitamin B1) deficiency, known as beriberi (Almeida et al. 2012). A case of human poisoning via combined inhalation and ingestion of the ionophore salinomycin showed that a dose of 1 mg kg^{-1} led to serious damage to skeletal muscles caused most likely by ATP depletion (Story and Doube 2004).

Whereas foodborne exposure to toxins occurs naturally through ingestion and consequent absorption in the digestive tract, exposure to toxins in indoor air most likely occurs through inhalation of airborne particles and semivolatile compounds. Acute effects can therefore be expected to center on the respiratory tract and mucous membranes. For example, the primary symptoms experienced by individuals in moisture-damage buildings tend to be flu-like, such as rhinitis, cough, sore throat, as well as irritation of the eyes and skin via air contact (Putus 2014). Mitochondrial toxins found in moisture-damaged buildings, such as acrebol (produced by *Acremonium exuviarum*), amylosin, cereulide, and valinomycin, have been shown to be cytotoxic to feline fetal lung cells (Andersson et al. 2005, Andersson et al. 2009, Mikkola et al. 2007). Cereulide and valinomycin produced by indoor air isolates also affect mitochondria in human lung cells (Andersson et al. 2005). These reports suggest that mitochondrial toxins may have direct effects on pulmonary functions.

1.5.2. Chronic effects

Mitochondrial dysfunction is implicated in many acquired conditions with rising incidence worldwide, such as cancer, diabetes, inflammatory bowel disease, neurodegenerative disorders such as Alzheimer's, Parkinson's and Huntington's diseases as well as pathological conditions of the heart and cardiovascular system, kidneys, liver, eyes, ears, gastrointestinal tract, and even psychiatric disorders such as depression and schizophrenia (reviewed by DiMauro et al. 2006, Neustadt and Pieczenik 2008, Novak and Mollen 2015). Toxic compounds which disrupt mitochondrial functions are known to affect at least the liver, cardiovascular system, skeletal muscles, kidneys, pancreas, and nervous system (Dykens and Will 2007, Eirin et al. 2014). For example, mitochondrial dysfunction is one of the main causes of drug-induced liver injury, which often prevents the approval of new pharmaceuticals and has also led to market withdrawals of clinical drugs (Labbe et al. 2008).

Long-term or repeated exposure to subacute levels of toxins leading to chronic symptoms is an area where knowledge is currently lacking (Rajkovic 2014). Pesticide exposure has been linked to the rising incidence of many chronic diseases, and one of the mechanisms implicated as a cause is mitochondrial damage, as many pesticide compounds inhibit mitochondrial functions, such as oxidative phosphorylation or fatty acid metabolism (Mostafalou and Abdollahi 2013). Microbially produced mitochondrial toxins exhibit similar effects on mammalian cells and could therefore also add to the burden of increasing chronic diseases. For example, low doses of cereulide have many toxic effects on pancreatic insulin-producing beta cells, suggesting that chronic exposure via staple foods to low amounts of cereulide may contribute to the worldwide rise in the incidence of diabetes, but *in vivo* studies to confirm this hypothesis have not yet been conducted (Vangoitsenhoven et al. 2015). Chronic effects of mitochondrial toxins may also follow from states of chronic inflammation caused by continued inflammasome activation (see section 1.5.3.). For example, chronic inflammation in the pulmonary tract can lead to the development of asthma, which is linked to exposure to damp indoor environments (Heseltine et al. 2009, IOM 2004, Putus 2014).

1.5.3. Immunotoxicity

Taking into account the multifaceted role of mitochondria in controlling the reactions of the innate immune system (section 1.3.2), it is not surprising that mitochondrial dysfunction has been shown to lead to stimulation of cytokine release and therefore activation of the innate immune system (reviewed by Elliott and Sutterwala 2015, Gurung et al. 2015). Of the toxic compounds presented in Table 2, α-hemolysin, Panton-Valentine leucocidin, pneumolysin, TcdA, TcdB, gramicidin, nigericin, and LPS have been shown to activate the NLRP3 inflammasome, and valinomycin has been reported to stimulate active cytokine IL-1β release from human monocytes (Allam et al. 2011, Elliott and Sutterwala 2015, Holzinger et al. 2012, Karmakar et al. 2015, Kebaier et al. 2012, Ng et al. 2010, Walev et al. 1995). In addition, the well-known mitochondrial respiratory chain inhibitor rotenone is also a known activator of this inflammasome (Zhou et al. 2011). Disruption of mitochondrial ion homeostasis by microbially produced ionophores could activate inflammasomes since, for example, calcium influx and potassium efflux have been shown to trigger activation and cytokine release (Elliott and Sutterwala 2015, Gurung et al. 2015, Muñoz-Planillo et al. 2013).

Mitochondrial toxins with immunotoxic effects could play a role especially in the symptoms caused by exposure to toxins in indoor air, since individuals experiencing ill-health effects in moisture-damaged buildings most often display symptoms of inflammation, i.e., activation of innate immunity (IOM 2004). Exaggerated immune responses following from overstimulation of the innate immune system, overactivation of inflammasomes, and consequent unrestrained release of cytokines can lead to a so-called cytokine storm, which has been shown to have devastating effects on the body (Clark 2007). For example, a cytokine storm in the lungs can lead to acute lung injury and continue to spread to other organs via a spillover effect through blood circulation, leading to tissue damage and possibly sepsis (Tisoncik et al. 2012). It is worth noting that there appears to be quite a lot of variability in the innate immune responses of individuals, bringing about differences in susceptibility to cytokine storms (Tisoncik et al. 2012). It is commonly known that in many cases of both food poisoning and symptoms following exposure to moisture-damaged indoor spaces, individuals exposed to the same causative factor react differently, making it difficult to find conclusive evidence of causal relationships.

1.6. Methods for the detection of mitochondrial toxins

In recent years, legislative pressure to avoid unnecessary animal testing in the European Union has highlighted the importance of developing reliable *in vitro* methods (ECHA 2014). A wide range of *in vitro* methods for detecting substances which affect mitochondrial functions have been developed, but none have as yet been standardized. Thus it is not possible to compare, for example, the sensitivities of the different methods on a general level. Examples of used bioassays are compiled in Table 3.

The bioassays presented in Table 3 can be used in two ways: to determine the mitochondriotoxic effects of known substances (e.g., mitochondrial toxicity testing as part of drug development) and to detect mitochondrial toxicity in samples of unknown chemical composition (i.e., searching for novel mitochondriotoxins). In the latter case, chemical analysis, such as high-performance liquid chromatography combined with mass spectrometry (HPLC-MS), is required for the purification and identification of the toxic substance(s). Figure 4 presents a simplified schematic diagram of the process which can be used to find and identify novel mitochondrial toxins. Chemical analyses can also be used to search for specific mitochondrial toxins within a sample of unknown composition if the chemical characteristics and structure of the toxins are known.

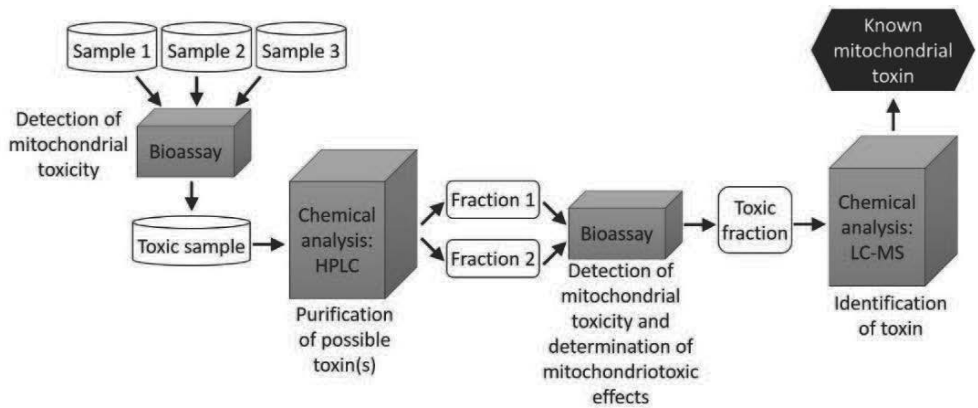

Figure 4. Simplified schematic diagram depicting the process of finding and identifying novel mitochondrial toxins.

Table 3. Examples of *in vitro* methods for detecting mitochondrial toxins and their modes of action.

Method	What is measured	Target cell / organelle	Reference
Blue native electrophoresis	Enzyme activity of Krebs cycle, oxidative phosphorylation and glycolysis	Tissue samples or intact mammalian cells	Han et al. 2013
Boar sperm motility inhibition (BSMI) assay	Sperm motility and $\Delta\Psi_m$	Viable porcine spermatozoa	Andersson et al. 1998a, Hoornstra et al. 2003
Cell respiratory control assay	Basal respiration, ATP turnover, proton leakage, coupling efficiency, maximum respiration rate, respiratory control ratio, spare respiratory capacity, non-mitochondrial respiration	Intact mammalian cells	Brand and Nicholls 2011
Confocal microscopy using fluorescent probes	$\Delta\Psi_m$ and calcium amounts	Intact mammalian cells and isolated mitochondria	Nieminen et al. 2008
DCFH-DA (2′,7′-dichlorodihydro-fluorescein diacetate) oxidation	Formation of reactive oxygen species	Isolated mitochondria	Freyre-Fonseca et al. 2011
Extracellular flux measurement	Oxygen consumption (mitochondrial respiration) and proton efflux (glycolysis)	Intact mammalian cells	Ferrick et al. 2008
Flow cytometry with fluorescent probes	$\Delta\Psi_m$, production of reactive oxygen species, calcium amounts, occurrence of apoptotic cell death	Intact or permeabilized mammalian cells	Christensen et al. 2013, Rottenberg 2002
Fluorescence-based oxygen uptake assay	Oxygen consumption	Intact mammalian cells	Hynes et al. 2006

Table 3. (cont.)

Method	What is measured	Target cell / organelle	Reference
Fluorescent probes and fluorescence microscopy	Visualization of mitochondria and DNA, $\Delta\Psi_m$, reactive oxygen species, mitochondrial permeability transition pore action	Intact mammalian cells and isolated mitochondria	Anon. 2010, Brand and Nicholls 2011
Glucose-consumption assay	Changes in glucose consumption	Intact mammalian cells	Ajao et al. 2015
HepG2 glucose/galactose assay	Mitochondrial ATP production	Human liver carcinoma cell line (HepG2)	Marroquin et al. 2007
High-throughput dual parameter assay	Extracellular acidification and oxygen consumption	Intact mammalian cells	Hynes et al. 2013
Immunocapture-based protein and enzyme activity assays	Enzyme activity of oxidative phosphorylation enzymes, mitochondrial protein levels and post-translational modifications of mitochondrial proteins	Mammalian cells or tissues or isolated mitochondria	Marusich et al. 2009, Nadanaciva 2008
Lactate production measurement	Lactate as an indicator of oxygen-independent energy production	Intact mammalian cells	Hoschele 2006
Lateral flow dipstick immunoassay for mtDNA dysfunction	Inhibition of mtDNA replication and/or mtDNA-encoded protein synthesis	Cell and tissue extracts, patient samples (e.g. blood)	Nadanaciva 2008
Metabolite profiling	Cellular energy metabolism	Intact mammalian cells	Balcke et al. 2011

Table 3. (cont.)

Method	What is measured	Target cell / organelle	Reference
Mitochondrial respiratory control assay	Changes in respiration rate in response to ADP addition	Isolated mitochondria	Brand and Nicholls 2011
Mitochondrial ToxGlo™ Assay (commecial kit: Promega, Madison, WI, USA)	Cell membrane integrity (necrosis) and ATP quantity	Intact mammalian cells	Arduengo 2012
Mouse embryonic fibroblast panel	Idiosyncratic mitochondrial toxicity as differences in mtDNA	Varying strains of mouse embryonic fibroblasts	Pereira et al. 2012
mtDNA assessment	mtDNA quantity	Intact mammalian cells	de Baar and de Ronde 2008
Oligomycin null-point test	Cessation of mitochondrial ATP functions	Intact mammalian cells	Brand and Nicholls 2011
Quenched-fluorescence oxygen sensing	Oxygen consumption	Intact mammalian cells or isolated mitochondria	Hynes et al. 2008
Respirometry using a Clark electrode	Mitochondrial respiration	Intact or permeabilized mammalian cells or tissues or isolated mitochondria	Gnaiger 2008
Spectrophotometric measurement	Activity of oxidative phosphorylation complexes I-IV	Mitochondrial fractions from tissues and cells	Birch-Machin 2008

1.6.1. Boar spermatozoa as indicators of mitochondrial toxicity

Boar spermatozoa are efficient as indicators of mitochondrial toxicity (Ajao et al. 2015, Hoornstra et al. 2003, Jääskeläinen et al. 2003, Vicente-Carrillo et al. 2015) and can be used to detect toxicity in food samples, such as rice and pasta, at concentrations relevant for human toxicity (Andersson et al. 1998a, Jääskeläinen et al. 2003). Boar spermatozoa are a close analogue to human sperm in many ways: e.g., they possess the sperm-specific Ca^{2+} channel CatSper (Vicente-Carrillo et al. 2014) which in human sperm is known to be activated by endocrine disrupting chemicals, causing elevated intracellular Ca^{2+} levels (Schiffer et al. 2014) which lead to mitochondrial dysfunction (Pandya et al. 2013). Substances toxic to boar spermatozoa can therefore be assumed to be toxic to human spermatozoa as well. For example, human cells and boar spermatozoa have been shown to be equally sensitive to cereulide (Jääskeläinen et al. 2003).

Boar semen is of higher and more consistent quality than human semen (Vicente-Carrillo et al. 2015) and more sensitive than e.g. bull semen (Jääskeläinen et al. 2003). It can be easily obtained from artificial insemination stations at low cost and in an animal-friendly manner. Boar spermatozoa can be especially useful for rapid toxicity screening of large sample amounts, as they enable the simultaneous detection of both loss of mitochondrial oxidative phosphorylation (indicated by loss of motility) and changes in the state of the mitochondrial membrane potential (using membrane potential sensitive dyes such as JC-1) (Hoornstra et al. 2003). Recent work by Vicente-Carrillo et al. (2015) showed that this combined type of analysis using boar spermatozoa paralleled the conventional test with human hepatoma cell line HepG2, often used in the pharmaceutical industry for *in vitro* screening of drugs, in detecting compounds causing human liver toxicity. However, the boar sperm assay gave results much faster than the HepG2 test (within 30 min vs. after 24 to 48 h), indicating that the boar sperm assay could be more user friendly (Vicente-Carrillo et al. 2015).

2. Aims of the study

The purpose of this work was to investigate the types and prevalence of mitochondrial toxins produced by microbes in indoor air of moisture-damaged buildings as well as food and study the effects of these toxins on mammalian cells to gain insight into the possible consequences of long-term human exposure to sublethal concentrations. The hypothesis was that exposure to mitochondrial toxins via various routes could be more common than previously thought and this continued exposure to sub-acute toxicity may have long-term harmful health effects.

Specific aims were to:

1. develop methods for the collection of toxic aerosols in indoor air and test the suitability of the boar sperm motility assay for assessing aerosol toxicity (Paper I),

2. specify the effects of the bacterial toxin amylosin, produced by bacteria isolated from both indoor air and food, on mammalian cells and mitochondria (Paper III), and

3. uncover the cause(s) of observed toxicity in grain samples collected by the Finnish Food Safety Authority EVIRA (Papers II and IV).

3. Materials and methods

All used materials and methods are described in the original papers (I-IV) which this thesis is based upon. Used methods are listed in Table 4. Used microbial strains are presented in Table 5 and used mammalian cell types in Table 6.

Table 4. List of methods used in Papers I-IV.

Method	Paper
Sampling	
Collection of indoor aerosols and dust	I
Isolation of bacterial strains	
Isolation from indoor dust	I
Isolation from grains	II, IV
Microscopy	
Light microscopy	I, II, IV
Fluorescence microscopy	II, III, IV
Detection of airborne compounds	
Flow-through chamber test	I
Conway chamber test	I
Analysis of chemical elements	
Inductively coupled plasma mass spectrometry (ICP-MS)	I
Investigation of conditions for toxin production	
Testing of cold tolerance of toxin production	II
Identification and characterization of bacterial strains	
Gram staining	II
Catalase and oxidase tests	II
Hydrolysis of starch	II
NaCl tolerance test	II
Penicillin susceptibility	II
Haemolysis test	II
Determination of growth temperature range	II
Growth on varying media	II
Whole-cell fatty acid analysis	II, IV
16S rRNA gene sequencing (whole or partial)	II, IV
Whole-cell DNA fingerprinting by ribopattern analysis	II, IV
Bacillus cereus and cereulide-specific PCR assays	II
Characterization service of DSMZ	I, IV
Extraction of toxins	
Extraction from electrostatic filters, dust samples and building materials	I
Crude extraction from bacterial biomass	II, IV
Preparation of semipurified extracts from bacterial biomass	I, II, III, IV
Extraction from grains	IV

Table 4. (cont.)

Identification, quantification and purification of toxins	
High-performance liquid chromatography with UV detection (HPLC-UV)	I, II, III
Reversed-phase high-performance liquid chromatography (RP-HPLC)	I, II, III
HPLC-electrospray ionization ion trap mass spectrometry (HPLC-ESI-IT-MS)	I, II, III, IV
Investigation of effects on mammalian cells	
Testing for toxicity in labware	I
Rapid boar sperm bioassay	I, II, IV
Boar sperm motility inhibition assay (BSMI assay)	I, II, III, IV
Detection of changes in membrane potential ($\Delta\Psi$) using the membrane potential responsive dye JC-1	II, III, IV
Detection of changes in glucose consumption (glycolysis)	II, IV
Detection of metabolic acidosis	II
Detection of mitochondrial depolarization in intact cells	II, IV
Evaluation of effects on proliferating cells	II
Detection of cytokines using enzyme-linked immunosorbent assays (ELISA)	III
Detection of cytokines using Western blotting	III
Detection of cytokine mRNA transcription	III
Detection of necrotic cell death (resazurin reduction assay and triple staining with propidium iodide, calcein AM, and Hoechst 33342)	IV
Measurement of potassium ion efflux from intact cells	III
Investigation of effects on microbial cells	
Assessment of growth inhibitory effects against bacteria and fungi	III
Investigation of effects on isolated rat liver mitochondria (RLM)	
Detection of mitochondrial swelling	II
Measurement of oxygen uptake	II
Measurement of membrane potential	II
Measurement of mitochondrial functions	II
Measurement of the kinetics of K^+ influx into mitochondria	II
Investigation of effects on lipid membranes	
Black lipid membrane (BLM) method for measurement of ionophoricity	II

Table 5. List of microbial strains used in Papers I-IV.

Identifier(s)	Origin	Reason for use	Reference	Paper
Bacterial strains				
Bacillus amyloliquefaciens 19b (HAMBI 2660)	Insulation material of a residential building in Finland with moisture problems	Producer of amylosin	Mikkola et al. 2004	I, II, III
B. amyloliquefaciens IAM 1521 (HAMBI 2718)	Source unknown; obtained from IAM Culture Collection, Tokyo, Japan	Comparison strain (amylosin non-producer)	unpublished	III
Bacillus cereus 7/pk4 (HAMBI 2711)	Indoor wall of a hospital facility in Finland with occupants complaining of building-related ill health symptoms	Producer of cereulide	Apetroaie et al. 2005	I
B. cereus F4810/72 (HAMBI 2454)	Cooked rice involved in a food poisoning incident, isolated in the UK by the Public Health Laboratory Service	Producer of cereulide	Häggblom et al. 2002, Turnbull et al. 1979	II, III
B. cereus ATCC 14579^T	Air in cow shed	Comparison strain (cereulide non-producer)	Frankland and Frankland 1887	II
B. cereus NS58 (HAMBI 2450)	Aseptic core drill sample 3.5 m from up the trunk of a live spruce tree (*Picea abies*) in Ruotsinkylä research forest of the Natural Resources Institute Finland	Producer of cereulide	Hallaksela et al. 1991	II, III, IV
Bacillus megaterium Ne10 (DSM 17641)	Water-damaged paperboard from a moisture-damaged building	Target strain for testing growth-inhibiting properties of amylosin	unpublished	III

Table 5. (cont.)

Identifier(s)	Origin	Reason for use	Reference	Paper
Bacterial strains				
Bacillus sp. OS16	Hay dust	Target strain for testing growth-inhibiting properties of amylosin	unpublished	III
Dietzia sp. MA147 (HAMBI 3595)	Water-damaged building material of a 21-year-old children’s daycare center in southern Finland with a history of repeated water damage and classified as a moisture problem building by health authorities	Target strain for testing growth-inhibiting properties of amylosin	unpublished	III
Mycobacterium murale MA113 (DSM 44340^{T})	Same as *Dietzia* sp. MA147	Target strain for testing growth-inhibiting properties of amylosin	Andersson et al. 1997, Vuorio et al. 1999	III
Paenibacillus polymyxa RS10 (HAMBI 3103)	Dust collected from the inside of a computer in a taxi station office in Kankaanpää, Finland, with occupant-reported building-related ill health symptoms	Toxic to boar spermatozoa (cause of toxicity unknown)	novel isolate	I
Paenibacillus tundrae E8a (HAMBI 3232)	Whole barley grains (cultivar Saana) from crop season 2001 cultivated in Southern Finland	Producer of paenilide	novel isolate	II
P. tundrae E8b	Same as *P. tundrae* E8a	Producer of paenilide	novel isolate	II
P. tundrae	Arctic tundra soil	Comparison strain (paenilide non-producer)	Nelson et al. 2009	II

Table 5. (cont.)

Identifier(s)	Origin	Reason for use	Reference	Paper
Bacterial strains				
Streptomyces griseus 10/ppi (DSM 41751)	Commercial bakery in Finland with workers suffering from building-related ill health symptoms	Producer of valinomycin	novel isolate	I
Streptomyces sp. (18 isolates)	Whole spring wheat grains and whole barley grains from crop seasons 2001 and 2002 cultivated in Southern and Western Finland	Producers of antimycin A and other substances toxic to boar spermatozoa	novel isolates	IV
Williamsia muralis 140/96 (DSM 44343^T)	Non-water-damaged gypsum wall liner of a sleeping room in a 21-year-old children's daycare center in southern Finland with a history of repeated water damage and classified as a moisture problem building by health authorities	Target strain for testing growth-inhibiting properties of amylosin	Andersson et al. 1997, Kämpfer et al. 1999	III
Fungal strains				
Aspergillus westerdijkiae pp2 (HAMBI 3333)	Dust collected over 3.5 months from the fine-dust filter of the shared central vacuum cleaner of a terraced two-family house in Lohja, Finland with reported building-related ill health symptoms	Producer of stephacidin B and avrainvillamide	novel isolate, identification by Mikkola et al. 2015	I
Aspergillus westerdijkiae KaIII (HAMBI 3335)	Aerosol collected by electrofiltration from a kindergarten in Tampere, Finland, with occupant-reported building-related ill health symptoms	Producer of stephacidin B and avrainvillamide	novel isolate, identification by Mikkola et al. 2015	I
Chaetomium globosum MTAV35 (HAMBI 3336)	Microbially damaged indoor building environment involved in a fatal human case	Target strain for testing growth-inhibiting properties of amylosin	unpublished	III

Table 6. List of mammalian cells used in Papers I-IV.

Cell type	Abbreviation or cell line code	Source	Reference	Paper
Human keratinocytes	HaCaT	Haartman Institute, Helsinki, Finland	Boukamp et al. 1988	III
Primary human macrophages	*N/A*	Differentiated from monocytes isolated from human buffy coats from Finnish Red Blood Service, Helsinki, Finland	*N/A*	III
Human primary peripheral blood mononuclear cells	PBMC	Isolated from human buffy coats from Finnish Red Blood Service, Helsinki, Finland	*N/A*	III
Murine neuroblastoma cells	MNA	Finnish Food Safety Authority EVIRA, Helsinki, Finland	*N/A*	II
Porcine spermatozoa	*N/A*	Figen Oy, Tuomikylä, Finland	*N/A*	I, II, III, IV
Porcine kidney tubular epithelial cells	PK-15	Finnish Food Safety Authority EVIRA, Helsinki, Finland	Echard 1974	II, IV

N/A = Not applicable

4. Results and discussion

This work consisted of two parts, one focusing on microbially produced toxic compounds found in moisture-damaged buildings and their effects on mammalian cells and the other aimed at determining novel food safety risks related to toxin-producing bacteria in stored grains. Papers I and III present research related to the first focus area, covering methods useful for the collection and toxicity assessment of indoor air aerosols and investigating the effects of the indoor air toxin amylosin on mammalian cells. Papers II and IV report on two studies related to previously unknown or underestimated food safety hazards: the discovery of a completely new peptide toxin produced by *Paenibacillus tundrae* isolated from barley grains and the finding of toxin-producing *Streptomyces* strains in barley and wheat grains.

4.1. Toxic compounds in moisture-damaged buildings

The work presented in Papers I and III concerns mitochondrial toxins in moisture-damaged indoor environments. Paper I focuses on method development including the BSMI assay, which was also used in all other Papers connected to this thesis work. Paper III presents a more detailed investigation into the effects of the indoor air toxin amylosin on mammalians and microbial cells.

4.1.1. Toxicity of indoor dusts and aerosols

Paper I describes and evaluates three different methods for the collection of indoor dusts and aerosols for toxicity testing: wiping and swabbing settled dust from surfaces, collecting dust from vacuum cleaners filters and dust bags, and electrostatic filtration. Using the inhibition of boar spermatozoa motility (BSMI assay) as an indicator, toxicity was found in samples collected from moisture-damaged indoor spaces with all three methods (Table 2 in Paper I). Aerosols collected by electrofiltration contained on average six times more ethanol/methanol soluble compounds than dusts collected by other means (Table 2 in Paper I), indicating that the electrostatic filters were efficient at collecting lipid-containing aerosolized substances.

The toxicity of the collected dusts and aerosols detected using the BSMI assay was clearly higher in samples from indoor environments where occupants reported ill health symptoms (Table 2 in Paper I and Figure 5). Although the sample size of the study was not sufficient for reliable statistical analysis, it would appear that there was a possible correlation between the ill health complaints and toxicity detected using the BSMI assay.

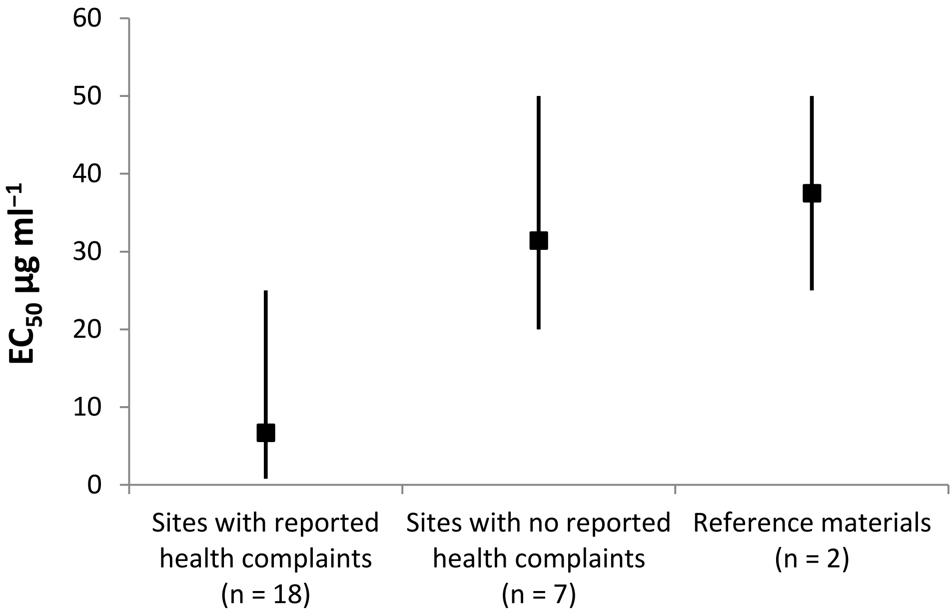

Figure 5. Range of toxicity of dust and aerosol samples detected using the BSMI assay. Toxicity is expressed as EC_{50} values, indicating the amount of ethanol or methanol-soluble compounds as µg per mg^{-1} of dust or of aerosol required for inhibiting the motility of >50% of the boar sperm cells within 4 d compared to vehicle control. Figure shows average (■) as well as range (vertical lines) for each sample group. Settled dust from a hay storage barn (accumulated over a period of >3 years) and substances from an unused electrostatic filter were used as reference samples. The figure is based on data presented in Table 2 of Paper I.

Of the collected dust and aerosol samples, 16 were cultured (13 from sites with reported health complaints and 3 from sites with no reported health complaints). Mixed microbial cultures obtained from 12 of the collected dust samples from sites with reported health complaints had EC_{50} values of <30 µg mg^{-1} within one day of exposure in the BSMI assay, indicating toxicity (Table 3 in Paper I). A total of 42 isolates obtained from these mixed cultures showed toxicity in the BSMI assay. As presented in Paper I, 11 of these isolates were identified as *Aspergillus westerdijkiae* (identification reported by Mikkola et al. 2015), *Bacillus amyloliquefaciens*, *Bacillus cereus*, *Bacillus simplex*, *Bacillus subtilis*, *Paenibacillus polymyxa*, and *Streptomyces griseus*.

All of these taxons have been previously reported from moisture-damaged indoor environments. *Aspergillus* is one of the most commonly found fungal genera in damp indoor spaces and building materials (Andersson et al. 1997, Heseltine et al. 2009, Verdier et al. 2014) as well as indoor dust (Rintala et al. 2012). *Aspergillus* species have also been linked to mycotoxins present in building materials (Tuomi et al. 2000). *Bacillus* species including *B. cereus*, *B. subtilis* and *B. amyloliquefaciens* have been reported to be common in dust and air in moisture-damaged buildings (Andersson et al. 1999), and toxin-producing *B. amyloliquefaciens* strains have been isolated from dust and insulation materials of moisture-damaged sites (Mikkola et al. 2004). *P. polymyxa* has been found in building materials in moisture-damaged buildings (Andersson et al. 1997). *S. griseus* and other *Streptomyces* spp. have been isolated from building materials (Andersson et al. 1997, Andersson et al. 1999) and settled dust (Andersson et al. 1998b) in moisture-damaged buildings. Overall, it has been suggested that the presence of *Streptomyces* in indoor spaces could indicate dampness and moisture damage (Rintala et al. 2004).

Four toxins were identified from the pure cultures which were toxic in the BSMI assay: amylosin (from *B. subtilis*), cereulide (from *B. cereus*), valinomycin (from *S. griseus*), and stephacidin B (from two strains of *Aspergillus westerdijkiae*). These compounds had a toxic effect at very low concentrations (EC_{50} values of ≤0.2 µg ml^{-1} after 3 to 4 day exposure) in the BSMI assay (Table 4 in Paper I). Amylosin, cereulide, and valinomycin collapse the potassium homeostasis of cells and mitochondria (Mikkola et al. 2007, Teplova et al. 2006), whereas the toxicity of stephacidin B is not due to a direct effect on mitochondria (Mikkola et al. 2015) but instead possibly linked to it binding to cysteine residues in proteins (Wulff et al. 2007), such as the cysteine-rich protein which affects the motility of spermatozoa (Hawthorne et al. 2006). Despite this difference in the mechanism of toxicity, these four toxins, and other toxic substances not yet identified, were detected by the BSMI assay, and could explain at least a part of the overall toxicity towards boar spermatozoa observed in the dust the aerosol samples.

Amylosin was first detected from *B. amyloliquefaciens* strains isolated from moisture-damaged buildings (Mikkola et al. 2004). Producers of amylosin, most closely affiliated with *B. subtilis* group species (Apetroaie-Constantin et al. 2009, Mikkola et al. 2004), as well as producers of cereulide, which belong to a specific subgroup of *B. cereus* (Agata et al. 1996, Ehling-Schulz et al. 2005), are both common in sick buildings (Andersson et al. 2005) and are also connected with food poisoning (Apetroaie-Constantin et al. 2009, Rajkovic 2014). Producers of valinomycin representing different species of *Streptomyces* were also previously reported in moisture-damaged buildings (Andersson et al. 1998b, Andersson et al.

2005, Täubel et al. 2011). Stephacidin B, however, has previously been reported only from an *Aspergillus ochraceus* strain isolated from clay in India (Qian-Cutrone et al. 2002). It is interesting that a producer of stephacidin B was found in two separate sampling sites (see Table 5 in this thesis), indicating the possibility that it might be present more commonly in "sick buildings", since species of *Aspergillus* are considered common contaminants of moisture-damaged sites (Heseltine et al. 2009).

4.1.2. Detecting indoor aerosolization and transfer of toxic compounds

Two methods for detecting the aerosolization of toxic compounds were examined in Paper I. Using a stainless steel flowthrough chamber, it was shown that measurable amounts of valinomycin, produced by a strain of *Streptomyces griseus*, was transferred via air to the stainless steel walls and the glass fiber exit valve filter of the chamber (Table 5 in Paper I). The transfer of valinomycin was detected by LC-MS analysis and the transfer of toxicity was confirmed using the BSMI assay. In addition, a Conway chamber was used to study toxic compounds emitted into the air from building materials. Boar spermatozoa motility was used to detect the transfer of toxicity via air (Table 6 in Paper I). Both approaches showed that boar spermatozoa can be used as indicators of aerosolized compounds toxic to mammalian cells.

The samples collected by electrofiltration (Table 1 in Paper I) contained a higher amount of lipophilic material compared to settled dust samples, indicating that vehicles other than dust can carry hydrophobic compounds in air. Recently, hydrophobic toxins such as communesins and chaetoglobosin were shown to be carried by water vapor (Salo et al. 2015). This is explained by the air-side of the air-water interface of water nanodroplets present in air which is very hydrophobic, suggesting that these droplets would attract non-polar compounds and could act as carriers (van Oss et al. 2005). The log K_{OW} of valinomycin is 4.49 (Teplova et al. 2006), indicating that it is a highly hydrophobic compound. Since acid proof stainless steel is a hydrophobic surface (Raulio et al. 2008), airborne water nanodroplets could be the vehicle which transferred valinomycin to the steel surfaces of the test chamber via air (Table 5 in Paper I). This type of transfer could explain why renovation of moisture-damaged buildings does not always lead to the disappearance of ill health symptoms in occupants (Rudblad et al. 2002, Sauni et al. 2015): toxic substances causing the symptoms may be transferred back into the building along with furniture and other items which could have "collected" hydrophobic toxic compounds in the moisture-damaged interiors prior to repair. There are no published reports definitively demonstrating this type of transfer, but official published guidelines and expert recommendations for the remediation of moisture-damaged indoor spaces state that it is often necessary to dispose of all dispensable items which have been exposed to the moisture-damaged space, as these items may cause a renewal of symptoms (Anon. 2011, Kärki and Öhman 2007). Overall, it has been stated that the effects of remediation of moisture-damaged buildings have not been studied sufficiently (Tischer and Heinrich 2013).

4.1.3. BSMI assay as a detector of toxicity

The results of the work presented in Paper I showed that the BSMI assay is useful as a method for detecting potential airborne toxicity and toxigenic microbes in indoor environments of moisture-damaged buildings. This method has previously been shown to be effective for detecting toxin-producing microbes and toxicity in moisture-damaged buildings and building materials from such sites (Andersson et al. 1997, Andersson et al. 1998b, Andersson et al. 2009, Mikkola et al. 2004, Peltola et al. 2004). However, the subjectivity of the assay readout, the unknown composition of the mixed toxic chemical load of indoor dusts, and the sometimes modest differences in the EC_{50} values of dusts from damaged vs. non-damaged buildings hampers the use of this method for comparing toxicities in buildings where inhabitants are exposed to unhealthy indoor air. Refinement of the assay as well as development of more representative sampling is still needed, but overall the method shows promise and could be used as a preliminary screening method in the search for indoor air toxicity.

In our work with boar spermatozoa, it was noted that there is a risk of false positive results due to toxicity leaching from plastic laboratory disposables, such as test tubes and centrifuge tubes as well as latex and vinyl gloves, into the vehicle substance (e.g. ethanol or methanol) (Paper I). Toxicity testing of laboratory disposables and media using human sperm for detecting embryonic toxicity is a common method of quality control used in *in vitro* fertilization laboratories (Claassens et al. 2000, Lierman et al. 2007). For example, the human sperm motility assay has been used to detect toxicity in gloves (Lierman et al. 2007) as well as syringes, filters and blood collection tubes (Claassens et al. 2000) used for *in vitro* fertilization. The results in Paper I highlight the importance of control samples and overall experiment quality control necessary in toxicity testing.

4.1.4. Effects of amylosin on mammalian and microbial cells

Results presented in Paper III show that amylosin, a heat-stable channel-forming toxin first identified from strains of *Bacillus amyloliquefaciens* isolated from moisture-damaged indoor spaces (Mikkola et al. 2004, Mikkola et al. 2007), had immunotoxic and potassium efflux-inducing effects on mammalian cells. Amylosin and its producer strain also displayed antagonistic effects on microbes.

4.1.4.1. Immunotoxic effects

The results in Paper III showed for the first time that amylosin stimulated the secretion of inflammatory cytokines IL-1β and IL-18 from LPS-primed human macrophages both in a biomass extract of strain *B. amyloliquefaciens* 19b (amylosin producer) and as a purified substance (Fig. 1 and 4 in Paper III). Upregulation of cytokine mRNA transcription and high levels (>2,000 pg ml^{-1}) of active cytokine secretion were induced within 2 h of exposure to nanomolar concentrations (50 ng ml^{-1}) of amylosin (Fig. 2 in Paper III).

This is the first report of a bacterial channel-forming ionophore toxin stimulating cytokine release from human primary macrophages. Other bacterial toxins reported to induce IL-1β and/or IL-18 secretion include nigericin from *Streptomyces hygroscopicus*, hemolysin and multifunctional repeat-in-toxin from *Vibrio cholerae* and *V. vulnificus*, cytolethal distending toxin from *Aggregatibacter actinomycetemcomitans*, community-acquired respiratory distress syndrome toxin from *Mycoplasma pneumoniae*, β-hemolysin from *Streptococcus agalactiae*, Panton-Valentine leucocidin and α-hemolysin from *Staphylococcus aureus*, TcdA and TcdB toxins from *Clostridium difficile*, antrax lethal toxin from *Bacillus anthracis*, gramicidin from *Brevibacillus brevis*, and valinomycin from *Streptomyces* spp. (Bose et al. 2014, Cordoba-Rodriguez et al. 2004, Gupta et al. 2014, Holzinger et al. 2012, Kebaier et al. 2012, Mariathasan et al. 2006, Ng et al. 2010, Shenker et al. 2015, Toma et al. 2010, Walev et al. 1995). In addition, the trichothecene mycotoxins roridin A, verrucarin A, and T-2 toxin have been shown to activate IL-1β and IL-18 secretion from human primary macrophages (Kankkunen et al. 2009). It is difficult to compare the results of these reports to the results presented in Paper III, as the used methods (e.g., varying exposure times, culture conditions, cell densities) and cell types (e.g., murine macrophage and human monocytic leukemia cell lines vs. human primary macrophages) are not standardized and most published results have not been obtained with pure substances. However, it can be seen that the amount of IL-1β (up to >30,000 pg ml^{-1} within 18 h) released by macrophages exposed to nanomolar concentrations of amylosin is higher than the IL-1β release induced by other toxins (varying from 40 to 13,000 pg ml^{-1}) (Bose et al. 2014, Cordoba-Rodriguez et al. 2004, Gupta et al. 2014, Holzinger et al. 2012, Kankkunen et al. 2009, Kebaier et al. 2012, Mariathasan et al. 2006, Ng et al. 2010, Shenker et al. 2015, Toma et al. 2010), indicating that amylosin is a highly potent activator of the NLRP3 inflammasome.

It is generally accepted that symptoms experienced by inhabitants of moisture-damaged buildings, such as asthma and other disorders linked to immune reactions, are caused by exposure to microbes, microbial cell components, and compounds produced by microbes, with the main exposure route being inhalation (Heseltine et al. 2009). The release of active inflammatory cytokines IL-1β and IL-18 following inflammasome activation caused by substances such as amylosin could well explain at least part of the wide variety of symptoms, as these cytokines participate in many ways in both local and systemic inflammation (Dinarello 2009). The link between inflammasome activation and pulmonary diseases appears especially strong, since chronic inflammation is considered a characteristic feature of these disorders (Hosseinian et al. 2015). Amylosin is a moderately lipophilic compound with a log K_{OW} between 3 and 4 (Mikkola 2006), so it is plausible that it could be aerosolized similarly as valinomycin (Paper I), dispersed via water vapor as has been reported for fungal toxins (Salo et al. 2015), and inhaled by inhabitants of indoor spaces contaminated by amylosin-producing bacteria. On coming into contact with alveolar macrophages, the inhaled amylosin could induce inflammasome activation and subsequent cytokine release, causing inflammation-type reactions such as asthma-related symptoms, which are reportedly more common among occupants of moisture-damaged indoor environments than the general population (Fisk et al. 2007, Mendell et al. 2011, Mudarri and Fisk 2007, Piipari and Keskinen 2005). A similar hypothesis has previously been presented in relation to trichothecene mycotoxins produced by *Stachybotrys chartarum*, a known contaminant of water-damaged buildings (Kankkunen et al. 2009).

4.1.4.2. Toxin-induced efflux of potassium ions

Exposure to low concentrations (≤5 µg ml^{-1}) of purified amylosin caused significant dose-dependent potassium ion efflux from the cytoplasm of human somatic cells and porcine spermatozoa to the extracellular fluid within minutes (Fig. 5 in Paper III). Intact human primary PBMC and human cultured keratinocytes (HaCaT), both suspended in a potassium-free medium at 24 °C, released 40% and 1%, respectively, of intracellular K^+ within 15 min of exposure to 5 µg ml^{-1} (PBMC) or 3.2 µg ml^{-1} (HaCaT cells) of amylosin. In the same exposure conditions, the intracellular K^+ stores of porcine spermatozoa (90 nmol per 54×10^6 sperm cells, i.e., circa 1.7 fmol per sperm cell) were completely depleted within 5 minutes of exposure to 0.5 µg amylosin ml^{-1}, which corresponds with approximately 25 K^+ ions passing through the cell membrane per molecule of amylosin per minute at a concentration of 5×10^6 molecules of amylosin per sperm cell. The efflux of potassium ions led to the depolarization of both the cytoplasmic membrane and the mitochondrial membranes in human keratinocytes (Fig. 6 in Paper III) and PBMC cells.

It has been suggested that the NLRP3 inflammasome is activated not by specific foreign molecules acting as danger signals but rather by generic stress signals produced by cells being exposed to detrimental compounds (Abderrazak et al. 2015). One such generic activator is potassium efflux, which by itself has been shown to induce activation of NRLP3 in macrophages (Muñoz-Planillo et al. 2013) and neutrophils (Karmakar et al. 2015). In addition, prior to the recognition of the inflammasome in 2002 (Martinon et al. 2002), work published regarding the activation of IL-1β release following exposure of human primary monocytes to the potassium efflux-inducing bacterial toxins nigericin, gramicidin, and valinomycin highlighted the importance of potassium ion movement in cytokine release (Walev et al. 1995). The results presented in Paper III support this potassium efflux theory, since the toxicity of amylosin is based on its functioning as an illegitimate potassium channel. In addition, mitochondrial damage caused by exposure to amylosin may further increased NLRP3 activation, explaining the continued rise in levels of released cytokines over time after exposure to amylosin (Fig. 2 in Paper III).

4.1.4.3. Antimicrobial activity

When co-cultured on agar plates, the amylosin-producing *B. amyloliquefaciens* strain 19b inhibited the growth of a toxigenic fungus, *Chaetomium globosum* MTAV35, and various gram-positive and gram-negative bacterial strains originating from moisture-damaged indoor environments (Fig. 7 in Paper III). In addition, purified amylosin inhibited the growth of a firmicute, *B. megaterium* Ne10, and an actinobacterium, *Dietzia* sp. MA147, in broth culture. Amylosin was 100-fold more potent as a growth inhibitor than the known bioactive lipopeptides produced by certain strains of *B. amyloliquefaciens* (fengycin, iturin, surfactin) or cereulide produced by *B. cereus* (Table 1 in Paper III).

The observed antimicrobial effects of amylosin may explain why this compound is produced by *B. amyloliquefaciens* 19b, which also produces the biosurfactant surfactin and the antifungal compound fengycin (Mikkola 2006, Mikkola et al. 2007). The microbiota of indoor dusts in moisture-damaged buildings is diverse (Andersson et al. 1999, Nilsson et al. 2004, Peltola et al. 2001a, Rintala et al. 2008), making such sites competitive environments. Thus, the ability to produce substances which can suppress the growth of other microbes may give the producer a competitive advantage. Strains producing amylosin could become dominant in moisture-damaged buildings offering suitable growth conditions and therefore pose a significant health risk for the occupants of such sites.

4.2. Bacterial toxins causing novel food safety hazards in stored grains

The grains samples studied in Papers II and IV, received from the Finnish Food Safety Authority EVIRA, were selected for the current study because they showed unexplained rapid toxicity in the BSMI assay (M. A. Andersson and M. Jestoi, unpublished results). The sampled grains contained mycotoxins, but the detected amounts of enniatins and beauvericin (Table 1 in Paper IV) did not explain the rapidly detected toxicity. Therefore, further investigation into the possible bacterial contaminants of the grains was conducted, which led to the finding of a novel bacterial toxin, paenilide, as well as the discovery of toxic *Streptomyces* strains producing antimycin A and unidentified toxin(s).

4.2.1. Paenilide, a novel bacterial toxin

Paenilide is a bacterial heat-stable depsipeptide toxin which acts as a potassium ion carrier similarl to cereulide. Paper II is the first report of this toxin. Due to its structural and mass spectral similarity to cereulide, paenilide could be considered a member of the growing family of cereulide isomers and congeners (Marxen et al. 2015a). However, paenilide appears to be the first cereulide-like toxin produced by a species other than *B. cereus* sensu lato.

The producers of paenilide were isolated from grains of barley (Figure 6) and identified as two strains of *Paenibacillus tundrae* based on 16S rRNA gene sequencing and biochemical characterization. Strain E8a produced larger amounts of paenilide than strain E8b. Cereulide has been shown to increase the survival of *Bacillus cereus* by acting as a potassium scavenger (Ekman et al. 2012). In a preliminary experiment examining the effect of cereulide on the growth rate of *P. tundrae* E8a conducted as described by Ekman et al. (2012), it appeared that the exponential growth phase of the paenilide-producer was delayed and ended at a lower cell density in a potassium-deficient medium, implying that the availability of potassium affected the growth of this strain (Ekman 2010 personal communication, unpublished results). When cereulide was added to the medium, the growth of the paenilide-producer slowed regardless of the potassium concentration of the medium, indicating that cereulide is more effective as a potassium scavenger than paenilide. It has been suggested that cereulide may give its producers a competitive advantage in potassium-scarce environments (Ekman et al. 2012). The production of paenilide may offer its producer the same type of competitive advantage, albeit with lower efficiency.

Figure 6. Grain of barley with *Paenibacillus tundrae* E8a growth on tryptic soy agar medium.

Although the optimal growth temperature for most paenibacilli is 28 to 40 °C (Priest 2009), some are psychrotolerant and can grow in pasteurized foods such as vegetable purées and milk at temperatures below 8 °C (Carlin et al. 2000, Fromm and Boor 2004, Guinebretiere et al. 2001, Huck et al. 2008, Ivy et al. 2012, Masiello et al. 2014, Moreno Switt et al. 2014, Patterson et al. 2012, Ranieri et al. 2009, Ranieri et al. 2012, Schmidt et al. 2012). The production of paenilide by strain *P. tundrae* E8a occurred at the same rate (20 to 50 ng of paenilide per mg of biomass wet wt) at temperatures varying from +5 °C to +28 °C. The strain also grew and produced paenilide at +37 °C, but the toxin yield appeared lower based on the results of the BSMI assay. Unlike cereulide-producing *B. cereus* (Delbrassinne et al. 2011, Häggblom et al. 2002, Thorsen et al. 2009), *P. tundrae* E8a produced paenilide also at cold temperatures and could, therefore, endanger the food safety of chilled foods. Paenibacilli have been reported from raw milk (Coorevits et al. 2008, De Jonghe et al. 2010, Huck et al. 2008, Ranieri et al. 2012) and many kinds of vegetables (Fangio et al. 2010). Viable paenibacilli have also been detected in ultra-high temperature (UHT) treated milk (Scheldeman et al. 2004) and raw milk heated up to temperatures of 130°C (te Giffel et al. 2002), indicating that *Paenibacillus* spores may match in thermal tolerance those of notoriously heat-resistant, cereulide-producing *B. cereus* (Carlin et al. 2006, Luu-Thi et al. 2014).

Paenibacilli are also common in food-packaging paperboard (Pirttijärvi et al. 1996, Pirttijärvi 2000, Raaska et al. 2002, Suihko and Stackebrandt 2003, Suominen et al.

1997, Suominen et al. 2003, Väisänen et al. 1991), where they most likely end up due to their widespread presence as spores in soil and as endophytes in plants (Zeigler 2013). Damage to the interior polyethylene coating of food packaging carton gives microbes access to moisture and nutrients, leading to microbial growth inside the paperboard (Suominen et al. 1997). Therefore, foodstuffs themselves, such as raw milk, as well as cardboard packaging, such as milk cartons, could both acts as sources of *Paenibacillus* contamination posing a food safety hazard for products with extended shelf lives, even in proper refrigerated storage conditions. Figure 7 summarizes the possible routes through which paenibacilli could contaminate chilled foods.

Extending the shelf life of chilled food products is of great interest in the global food market, and secondary antimicrobial metabolites of paenibacilli have been investigated as a method for improving the microbiological safety of foods (Gerst et al. 2015, Girardin et al. 2002, Piuri et al. 1998, Stern et al. 2005, Yang et al. 2013). However, based on the results presented in Paper II of this thesis, it appears that novel food safety hazards could be connected to both unintentional and intentional addition of paenibacilli to chilled processed foods with extended shelf lives.

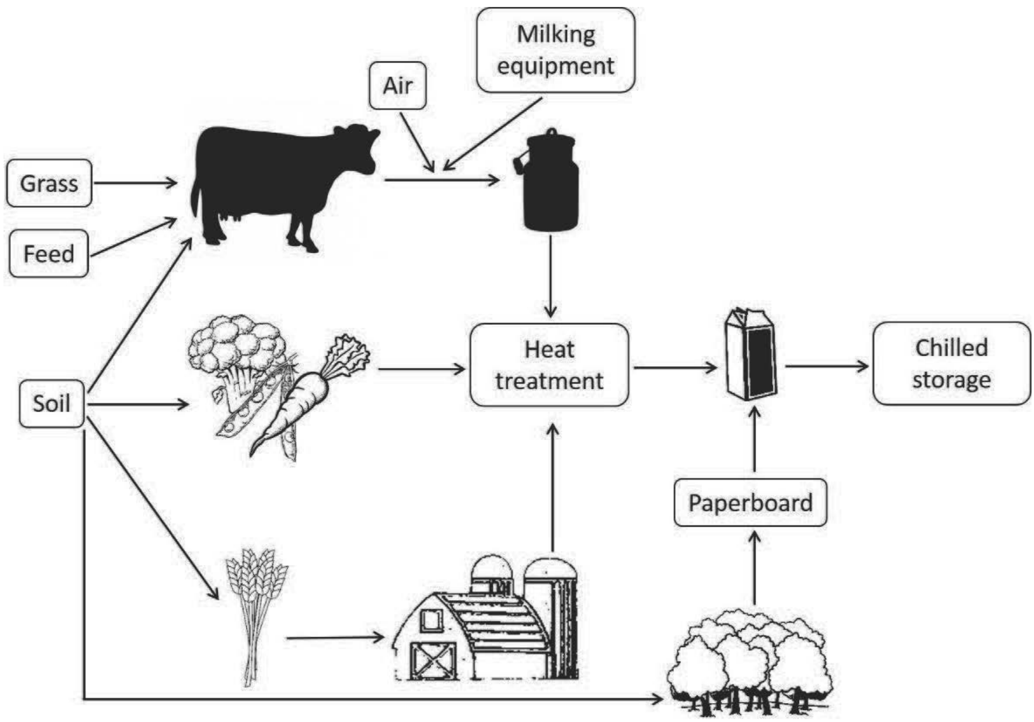

Figure 7. Potential routes through which *Paenibacillus* spp. could contaminate chilled foods.

4.2.1.1. Chemical characterization of paenilide

Paenilide consists of two components, named paenilide and homopaenilide, which could be separated from each other by HPLC fractionation. The molecular masses of paenilide (1,152 Da) and homopaenilide (1,166 Da) were identical to those of cereulide (Mikkola et al. 1999, Pitchayawasin et al. 2004) and homocereulide (Pitchayawasin et al. 2004, Wang et al. 1995), respectively, indicating they might represent novel members of the rapidly extending chemodiversity of cereulide (Marxen et al. 2015a). However, the retention times of the paenilide compounds were longer (+4 min), indicating more hydrophobic structures than those of the cereulide compounds. The mass fragmentation patterns of paenilide and homopaenilide were also identical to those of cereulide and homocereulide, respectively, but the higher hydrophobicity of the paenilide compounds indicated that they might contain three (paenilide) or four (homopaenilide) 2-hydroxy-3-methylpentanoic acid residues (*O*-Ile) instead of the three or four 2-hydroxyisocaproic acid residues (*O*-Leu) present in cereulide and homocereulide (Pitchayawasin et al. 2004), respectively. This structural difference implies that paenilide may be produced by synthetases encoded by genes independent of cereulide synthetases. This is supported by the fact that the paenilide-producing strain *P. tundrae* E8a tested negative for the cereulide synthetase gene *cesB* (Paper II).

Cereulide is of growing concern as a cause of food poisoning. Reports of the European Food Safety Authority (EFSA) show that the number of reported food-borne outbreaks with *Bacillus* toxins as the causative agent increased by 190% from 2010 to 2014 (99 outbreaks in 2010 vs. 287 outbreaks in 2013), while the total reported amount of outbreaks caused by bacterial toxins rose by 82% (461 outbreaks in 2010 vs. 840 outbreaks in 2014), with *Bacillus* toxins accounting for 34% of all bacterial toxin-related outbreaks in 2014 (EFSA and ECDC 2012, EFSA and ECDC 2015). It is not reported whether this rise is indicative of truly higher incidence or a result of increased awareness and improved detection methods. However, cereulide-producing strains of *B. cereus* have recently been shown to be more prevalent and present in more types of foods than previously assumed (Messelhausser et al. 2014). The detection and quantification of cereulide and cereulide-like compounds is thus of increasing importance. An ISO-EN standard for the quantitative analysis of cereulide in foods using LC-MS/MS (ISO/DIS 18465) is currently under development (status 30.12.2015; http://www.iso.org/iso/home/store/catalogue_tc/) and a method for the combined quantification of multiple cereulide variants has recently been developed (Marxen et al. 2015b). The finding of paenilide, the first cereulide-like toxic compound produced by bacteria other than *B. cereus* sensu lato, pinpoints that the developed methods should be versatile and specific enough to detect and differentiate between various cereulide variants and possible as yet unknown cereulide-like compounds.

4.2.1.2. Effects of paenilide on mammalian cells and mitochondria

The effect of paenilide (both compounds as a mixture) on mammalian cells was studied using porcine spermatozoa and kidney tubular epithelial cells (PK-15) as well as murine neuroblastoma (MNA). Exposure to subnanomolar amounts of paenilide led to various toxic effects in the test cells: loss of motility in the sperm cells, depolarization of mitochondria in sperm (Figure 8) and PK-15 cells, hyperpolarization of the plasma membrane in sperm cells (Figure 8), accelerated glucose consumption and metabolic acidosis in PK-15 cells, as well as cytolysis in PK-15 and MNA cells (Table 1 in Paper II). The toxicity equaled that of cereulide and homocereulide, indicating that low doses of paenilide could seriously damage human health, making paenilide a possibly potent food poisoning agent and bearing risks similar to those described for cereulide (Vangoitsenhoven et al. 2014, Vangoitsenhoven et al. 2015).

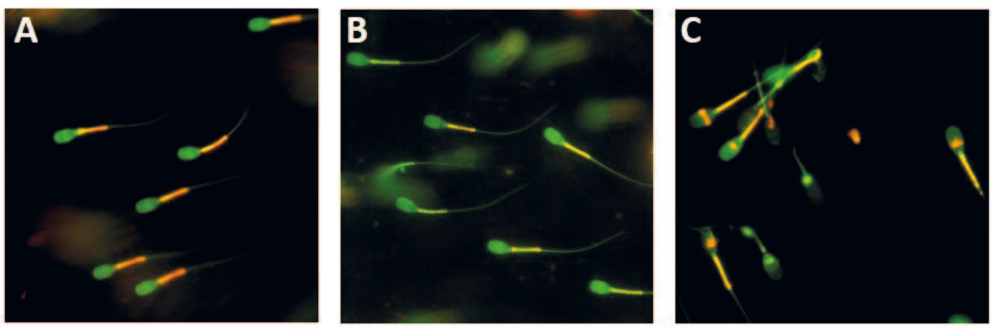

Figure 8. Epifluorescence micrographs of boar spermatozoa displaying effects of exposure to paenilide. Epifluorescence micrographs of boar spermatozoa displaying effects of exposure to paenilide. The sperm cells were stained with the membrane potential-responsive fluorogenic dye JC-1, which fluoresces orange when membrane potential ($\Delta\Psi$) is high (>140 mV) and green when $\Delta\Psi$ is low (<100 mV). **Panel A:** Cells exposed to vehicle only (methanol) for 1 d. **Panel B:** Cells exposed to 0.4 ng ml^{-1} paenilide for 1 d with depolarized mitochondria in the mitochondrial sheath (diminished orange fluorescence compared to Panel A). **Panel C:** Cells exposed to 1.3 ng ml^{-1} paenilide for 1 d displaying hyperpolarization in the head piece (orange fluorescence) and depolarization in the mitochondrial sheath (diminished orange fluorescence compared to Panel A).

In isolated rat liver mitochondria, paenilide acted as a potassium ionophore, which became saturated with K^+ ions at a potassium ion concentration equivalent to that of blood plasma (4 mM) (Figure 7 in Paper II), and caused an influx of potassium ions, leading to swelling, a loss of mitochondrial membrane potential, uncoupling of oxidative phosphorylation and loss of respiratory control (Figures 3, 4, 5, and 6 in Paper II). Tests using black lipid membranes confirmed that paenilide acted as a potassium-specific carrier. These results showed that the detected toxic effects of paenilide on mammalian cells are explained by its functioning as a lipophilic cationic compound transporting K^+ ions across cytoplasmic and mitochondrial membranes, similarly as cereulide and valinomycin (Mikkola et al. 1999, Teplova et al. 2006).

Paenilide appears to be the first reported compound with high toxicity to mammalian cells produced by a member of the genus *Paenibacillus*. *P. larvae*, a significant honey bee pathogen, produces several toxic compounds with different modes of action which have been shown to have antifungal and antilarval effects (Fünfhaus et al. 2013, Krska et al. 2015, Schild et al. 2014, Sood et al. 2014), and a strain of *P. polymyxa* has been reported to produce antifungal, insecticidal and herbicidal compounds (Zhao et al. 2011), but no report, other than Paper II of this thesis, of toxins relevant for mammalian health has so far been published. Traditionally, paenibacilli are not considered to cause any health risks. However, several species of *Paenibacillus*, including *P. larvae* and *P. polymyxa*, have been reported to cause bacteremia and pseudobacteremia in humans (Ko et al. 2008, Nasu et al. 2003, Noskin et al. 2001, Ouyang et al. 2008, Reboli et al. 1989, Rieg et al. 2010, Roux and Raoult 2004, Teng et al. 2003), indicating that they might be opportunistic pathogens. However, toxin production was not reported in any of the documented cases.

4.2.2. Toxin-producing *Streptomyces* in cereal grains

In the work presented in Paper IV, healthy-looking grains of barley and spring wheat, toxic in the BSMI assay, were found to harbor toxin-producing *Streptomyces* spp. The strains were isolated from randomly picked grains cultured on plates of tryptic soy agar. Figure 1 in Paper IV displays examples of grains with *Streptomyces* growth.

The isolates were identified and grouped based on whole-cell fatty acid analysis, ribosomal DNA fingerprinting, and 16S rRNA gene sequencing. Eight isolates from the barley sample were identified as members of the *Streptomyces albidoflavus* group and ten isolates from the spring wheat sample as *Streptomyces* sp., most closely related to *S. sedi*. Of the eight barley isolates, six produced detectable amounts of antimycin A (Table 3 in Paper IV), a heat-stable macrolide antibiotic with no current clinical use due to its high toxicity. Quantifiable amounts of antimycin A were also detected in a subsample of the barley grains prior to cultivation. Eight wheat isolates were highly toxic in the BSMI assay, but no known toxic substance was identified. These results show that streptomycetes may pose a novel threat for food and feed safety of stored grains. It is interesting to note that streptomycetes producing bioactive ionophoric compounds are being examined as possible biocontrol agents for controlling plant pathogens (Silva et al. 2014). If this type of use were to become common, the toxicity of these compounds towards mammalian cells, however, would need to be taken into account.

The toxicity of antimycin A towards porcine kidney epithelial (PK-15) cells was compared to that of other grain-associated mitochondrial toxins, namely cereulide, paenilide, and enniatin B. As presented in Paper IV, antimycin A was more toxic than the mycotoxin enniatin B but less toxic than cereulide or paenilide. For all four toxins, mitochondrial damage (detected as loss of $\Delta\Psi_m$ and acceleration of glucose consumption) was observed at exposure concentrations lower than those triggering necrotic cell death (Table 4 in Paper IV). The acceleration of glucose consumption in conjunction with the loss of $\Delta\Psi_m$ points to the upregulation of glycolysis. Many types of mammalian cells are capable of upregulating ATP production via glycolysis in the absence of oxidative phosphorylation, a phenomenon known as the Crabtree effect (Lunt and Vander Heiden 2011). When glucose is available, these cells are less susceptible to mitochondrial damage than cells relying on oxidative phosphorylation (Marroquin et al. 2007). This explains why the PK-15 cells survived at low exposure concentrations despite mitochondrial dysfunction (Paper IV).

However, not all cell types are able to survive solely on glycolytically produced ATP: for example, pancreatic beta cells lack this option and consequently become deprived of ATP if oxidative phosphorylation is blocked (Cantley et al. 2010, Maechler et al. 2006, Sener and Malaisse 1987). Upon exposure of murine pancreatic beta cells (MIN6) to cereulide, antimycin A, enniatin B, or the antibacterial preservative triclosan, loss of $\Delta\Psi_m$ and cell death occurred at approximately the same exposure concentration without a detectable increase in glucose consumption (Ajao et al. 2015, Hoornstra et al. 2013, Kruglov et al. 2009, Tonshin et al. 2010, Vangoitsenhoven et al. 2014, and personal communication Maria A. Andersson 2014). Cell death following exposure to cereulide was reported apoptotic at extremely low exposure concentrations (≤ 0.5 ng ml^{-1}) (Vangoitsenhoven et al. 2014) and necrotic at highly mitochondriotoxic concentrations (>0.4 µg ml^{-1}) (Hoornstra et al. 2013). Apoptotic cell death requires ATP, so cells relying solely on mitochondria for energy production undergo necrotic cell death once mitochondrially produced ATP is depleted (Leist et al. 1997). As can be seen in Figure 9, MIN6 cells exposed to valinomycin underwent necrotic cell death at the same concentration where mitochondria became depolarized, whereas cell types capable of switching to glycolytic ATP production (HaCaT and PK-15 cells) remained intact even when their mitochondria were depolarized. Similar results have been obtained with exposure to antimycin A, cereulide, enniatin B, myxothiazol, and triclosan (Ajao et al. 2015, Hoornstra et al. 2013, Kruglov et al. 2009, Tonshin et al. 2010, and personal communication Maria A. Andersson 2014). Necrotic cell death triggers inflammatory responses by activating inflammasomes, which can cause tissue damage as well as accelerate the onset of both type 1 and type 2 diabetes (Rock et al. 2011). Mitochondrial dysfunction has directly been implicated in pancreatic damage connected to both type 1 and type 2 diabetes (Maechler et al. 2006, Sivitz and Yorek 2010), the incidences of which are increasing worldwide (Canivell and Gomis 2014). Mitochondrial function is central in controlling insulin secretion from pancreatic beta cells by coupling glucose recognition to insulin secretion (Maechler et al. 2006). Continued low-dose exposure to heat-stable lipophilic mitochondrial toxins via staple foods such as grains could therefore explain at least part of the increase diabetes incidence, as has been suggested for cereulide (Vangoitsenhoven et al. 2015).

The findings reported in Paper IV indicate that streptomycetes could pose a hitherto unidentified food safety risk. *Streptomyces* are ubiquitous in soil and common as endophytic symbionts or parasites in various types of plants (Seipke et al. 2012). They are well known as producers of secondary metabolites, many of which have bioactive properties (Berdy 2012). Even though many published studies report on the presence of streptomycetes in staple food crops (see references in Paper IV), little research concerning food safety risks related to streptomycetes is found in published literature. *Streptomyces* spp. are known to grow in media with even lower moisture levels than those required by most grain contaminating fungi, such as Fusarium, indicating that conditions preventing fungal growth and mycotoxin production cannot necessarily be assumed to control the growth and toxin production of *Streptomyces*.

Antimycin A is not toxic to plants, as plant mitochondria can bypass the cytochrome b-dependent bc_1 respiratory complex using an alternative oxidase which is unaffected by antimycin A (Millar et al. 2011). Thus, contamination cannot be detected based on grain or plant health. The possible connection between food contaminated with secondary metabolites of streptomycetes and the increasing incidence of diabetes, for example, is not as far-fetched as it may seem: known diabetogenic drugs, such as streptozotocin, bafilomycin A1, and concanamycin A, all produced by *Streptomyces* spp., have been proposed as environmental promoters of type 1 and 2 diabetes (Bodin et al. 2015, Hettiarachchi et al. 2006, Myers et al. 2003), and maternal exposure to macrolide antibiotics other than antimycin A has been shown to increase the type I diabetes risk of the unborn child (Kilkkinen et al. 2006).

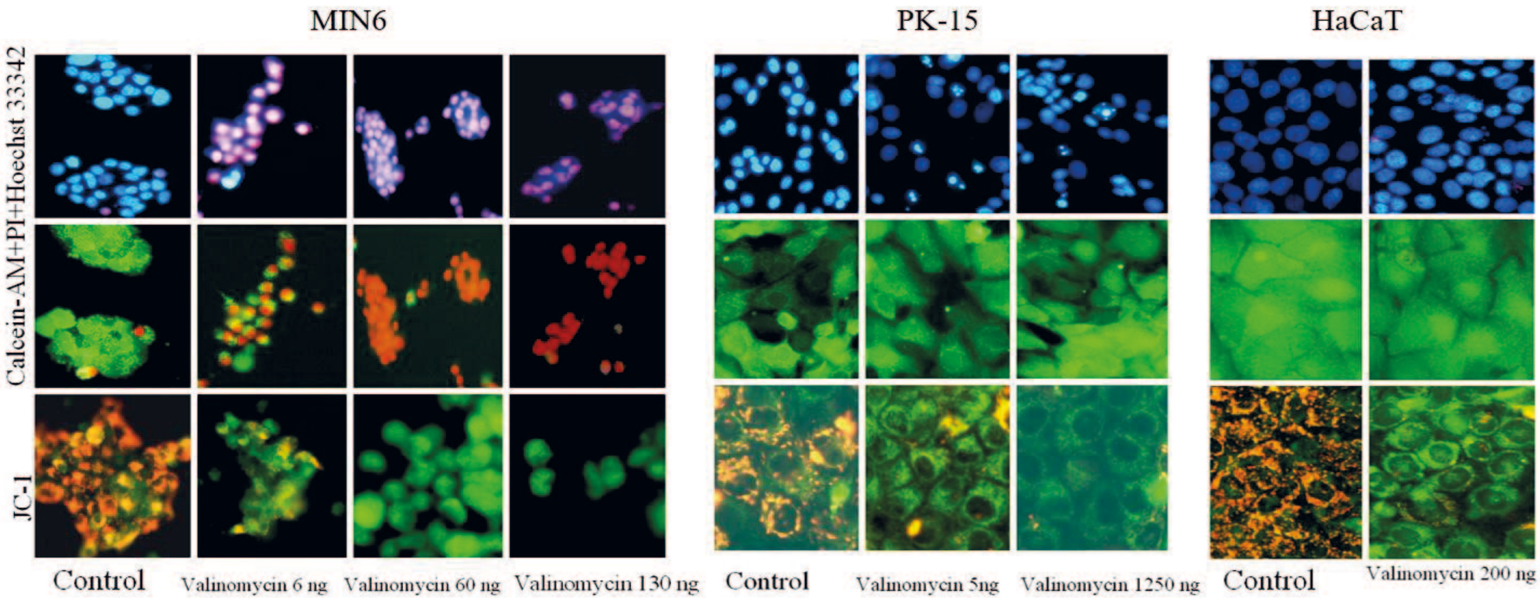

Figure 9. Epifluorescence micrographs of mammalian cells displaying effects of exposure to valinomycin.Murine pancreatic beta cells (MIN6), porcine kidney tubular epithelial cells (PK-15), and human keratinocytes (HaCaT) were stained with JC-1 (bottom row) to detect mitochondrial depolarization, calcein-AM and propidium iodide (middle row) to detect cell viability, and with Hoechst 33342 (top row) to detect intact nuclei. The membrane potential-responsive fluorogenic dye JC-1 fluoresces orange when membrane potential ($\Delta\Psi$) is high (>140 mV) and green when $\Delta\Psi$ is low (<100 mV). Orange fluorescence within the control cells thus indicates mitochondria with normal $\Delta\Psi$m ($\geq$160 mV), whereas lack of orange fluorescence after exposure to valinomycin indicates mitochondrial depolarization in all three cell types. Only MIN6 cells underwent necrosis following exposure to valinomycin, indicated by the red fluorescence in the middle row and the pink fluorescence in the top row, both due to the cells becoming permeable to propidium iodide. PK-15 and HaCaT cells remained intact despite exposure to valinomycin and mitochondrial depolarization. Pictures courtesy of Maria A. Andersson, University of Helsinki.

4.3. Mammalian cell toxicity of microbially produced mitochondrial toxins

The microbial toxins studied in this work represent chemically diverse compounds produced by different microbes, but they share the ability to cause mitochondrial dysfunction, albeit in various ways, and are produced by microbes capable of forming spores, i.e., capable of surviving in various less than optimal conditions. Mitochondrial toxicity leading to, e.g., liver failure and cardiovascular issues is the most common reason for market withdrawal of pharmaceuticals (Dykens and Will 2007). In addition, mitochondrial damage has been linked to pathological conditions in many different human organs, such as the pancreas, heart, liver, kidneys and nervous system (Eirin et al., 2014).

As can be seen from the results compiled in Table 7, the compounds studied in this thesis have toxic effects on many types of mammalian cells. In addition, cereulide, valinomycin, amylosin, and enniatin B can inhibit both bacterial and fungal growth (Paper III of this thesis and Ladeuze et al. 2011, Park et al. 2008, Roig et al. 2014, Tempelaars et al. 2011), possibly giving producers a competitive advantage in various environments and increasing the risk of exposure.

The results presented in this thesis were obtained with pure compounds under laboratory conditions. In reality, we are repeatedly exposed to more than one compound at any given time. The possible synergistic or additive effects of chronic multi-compound exposure via food and indoor air are currently poorly understood. Studies on the effects of a Northern contaminant mixture (a mix of organic and inorganic environmental contaminants found in the blood of Canadian Inuits) have shown adverse effects on human coronary artery endothelial cells, pancreatic MIN6 cells, and rat liver health, indicating a possible connection to the increased incidence of cardiovascular disease, diabetes, and non-alcoholic fatty liver disease among the exposed populations (Florian et al. 2013, Mailloux et al. 2014, Mailloux et al. 2015). The combined effects of exposure to multiple mitochondrial toxins via food and indoor air may have similar cumulative effects and therefore be more detrimental to human health than exposure to single compounds. It is worth noting that two of the studied mitochondrial toxins, cereulide and amylosin, have been found in both food and moisture-damaged indoor environments (Apetroaie-Constantin et al. 2009, Mikkola et al. 2004, Rajkovic 2014), indicating that exposure to a single compound could also occur through a combination of several sources.

Table 7. Effects of the mitochondrial toxins studied in this thesis on mammalian cells and mitochondria. Results are compiled from the papers presented in this thesis and results available in published literature.

	Mitochondrial toxin						
Toxicity endpoint	Amylosin	Antimycin A	Cereulide	Enniatin B	Paenilide	Valinomycin	References
Cytotoxicity (cell death)	+	+	+	+	+	+	II, IV, a, b, c, d, e
Potassium ion efflux from mammalian cells	+		+	+			III, e, f
Collapse of plasma membrane potential	+		+	+	+		II, III, a, e, g
Detrimental increase of glucose consumption		+	+	+	+	+	II, IV, h
Metabolic acidification of growth medium			+		+		II
Activation of cytokine release from macrophages	+	+		+			III, i, j
Loss of motility of boar spermatozoa	+	+	+	+	+	+	I, II, III, IV, a, c, g, h, k, l, m
Depolarization of mitochondria inside cells	+	+	+	+	+	+	II, IV, k, a, b, c, e, g, m, h
Depolarization of isolated mitochondria	+		+		+	+	II, a, n
Swelling of mitochondria	+		+	+	+	+	II, l, a, d, e
Uncoupling of oxidative phosphorylation	+		+		+		II, a

+: toxic effect observed / reported (empty cells indicate that information was not available)

References: I-IV Papers in this thesis; a) Mikkola et al. 2007, b) Hoornstra et al. 2013, c) Andersson et al. 2005, d) Vangoitsenhoven et al. 2014, e) Tonshin et al. 2010, f) Andersson and Salkinoja-Salonen 2005, g) Hoornstra et al. 2003, h) Mikkola et al. 2015, i) Jabaut et al. 2013, j) Gammelsrud et al. 2012, k) Jääskeläinen et al. 2003, l) Andersson et al. 1998a, m) Apetroaie-Constantin et al. 2009, n) Mikkola et al. 1999.

5. Conclusions

The conclusions based on the work presented in this thesis are:

1. Chronic exposure via food or indoor environments to sub-lethal concentrations of mitochondrial toxins produced by microbes may be more common than has been understood so far. We may be exposed to a multitude of microbially produced mitochondrial toxins, the synergistic and/or additive effects of which are currently unknown. Chronic exposure to multiple toxicants may explain at least part of the increasing worldwide incidence of conditions such as diabetes, asthma, allergies, cardiovascular diseases, and autoimmune neurological disorders.

2. Metabolites produced by spore-forming bacteria able to withstand adverse environmental conditions may pose a threat for human health. This work showed that spore-forming, toxin-producing bacteria belonging to the genera *Bacillus*, *Paenibacillus*, and *Streptomyces* can be present in food as well as indoor environments.

3. Stored cereal grains can contain bacterial toxins in addition to mycotoxins. This type of food safety hazard is not currently recognized by legal food or feed safety requirements.

4. The finding of paenilide, a cereulide-like heat-stable toxin produced by *Paenibacillus tundrae* even at chilled temperatures, presents a novel food safety hazard and shows that the genus *Paenibacillus* can no longer be considered harmless for human health.

5. Streptomycetes growing in stored cereal grains can produce toxic secondary metabolites, such as shown in this thesis for antimycin A, bearing risks for food and feed safety. Due to the heat-stability of such metabolites, subsequent food processing steps are not sufficient to prevent the effects harmful to human and animal health.

6. Amylosin, a mitochondrial toxin found in both moisture-damaged indoor environments and in food, directly affects cells of the human innate immune system, causing active cytokine release by most likely activating the NLRP3 inflammasome. This reaction could explain some of the autoinflammation-like symptoms experienced by inhabitants of moisture-damaged buildings where amylosin-producing bacteria are present.

7. Amylosin, similarly as cereulide and valinomycin, has both antifungal and antibacterial effects, which may therefore give bacteria producing these toxins a competitive advantage and promote their dominance in some moisture-damaged environments.

8. The BSMI assay is an efficient and inexpensive method for rapid detection of mitochondrial toxicity of both aerosolized compounds and food stuffs. Combined with the collection of toxic aerosols, the BSMI assay may be useful as a screening method for airborne toxicity in moisture-damaged indoor environments.

The findings of this thesis strongly support a recent call (Rajkovic 2014) for further research into the effects of repeated low-dose exposure as well as mixed simultaneous exposure to multiple compounds from one or more sources. Toxicity studies have traditionally focused on determining exposure threshold values at which acute and/or lethal symptoms appear and establishing safety limits based on these threshold values. In general, and especially when it comes to microbial toxins, less attention has been focused on examining the effects of chronic and sub-acute exposure. The standardized methods available for toxicity testing have mainly been developed for investigating the direct dose-response effects of exposure to inorganic chemicals, not the cumulative or multiplicative results of exposure to biochemical compounds, making it more challenging to study the toxicity of microbially produced compounds with these methods.

This thesis also highlights the need for continuous vigilance and increased awareness in the food sector of the hazards and risks related especially to spore-forming microbes contaminating foods. Known risks as well as the existence of so far unknown potential hazards should be, for example, considered in the development of novel food processing techniques aimed at shelf-life extensions.

In addition, the presented research indicates that bacteria and bacterial compounds may play a more significant role as causative agents of health problems experienced by inhabitants of moisture-damaged buildings than has thus far been understood. The majority of research concerning the microbial causes of building-related ill health symptoms has focused on fungi as causative agents. However, it appears that more attention should be paid also to bacteria and especially bacterial airborne toxins.

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References

Abderrazak, A., Syrovets, T., Couchie, D., El Hadri, K., Friguet, B., Simmet, T. and Rouis, M. 2015. NLRP3 inflammasome: from a danger signal sensor to a regulatory node of oxidative stress and inflammatory diseases. Redox Biol. 4: 296-307.

Abraham, A. K., Flatmark, T., Tangeras, A. and Pihl, A. 1982. Inhibition of mitochondrial protein synthesis and energy coupling by fragment A of diphtheria toxin. Eur. J. Biochem. 123(1): 201-207.

Agata, N., Mori, M., Ohta, M., Suwan, S., Ohtani, I. and Isobe, M. 1994. A novel dodecadepsipeptide, cereulide, isolated from *Bacillus cereus* causes vacuole formation in HEp-2 cells. FEMS Microbiol. Lett. 121(1): 31-34.

Agata, N., Ohta, M., Mori, M. and Isobe, M. 1995. A novel dodecadepsipeptide, cereulide, is an emetic toxin of *Bacillus cereus*. FEMS Microbiol. Lett. 129(1): 17-20.

Agata, N., Ohta, M. and Mori, M. 1996. Production of an emetic toxin, cereulide, is associated with a specific class of *Bacillus cereus*. Curr. Microbiol. 33(1): 67-69.

Ajao, C., Andersson, M. A., Teplova, V. V., Nagy, S., Gahmberg, C. G., Andersson, L. C., Hautaniemi, M., Kakasi, B., Roivainen, M. and Salkinoja-Salonen, M. 2015. Mitochondrial toxicity of triclosan on mammalian cells. Toxicology Reports 2: 624-637.

Akhtar, S., Sarker, M. R. and Hossain, A. 2014. Microbiological food safety: a dilemma of developing societies. Crit. Rev. Microbiol. 40(4): 348-359.

Allam, R., Darisipudi, M. N., Rupanagudi, K. V., Lichtnekert, J., Tschopp, J. and Anders, H. J. 2011. Cutting edge: cyclic polypeptide and aminoglycoside antibiotics trigger IL-1beta secretion by activating the NLRP3 inflammasome. J. Immunol. 186(5): 2714-2718.

Almeida, M. I., Almeida, N. G., Carvalho, K. L., Gonçalves, G. A., Silva, C. N., Santos, E. A., Garcia, J. C. and Vargas, E. A. 2012. Co-occurrence of aflatoxins B_1, B_2, G_1 and G_2, ochratoxin A, zearalenone, deoxynivalenol, and citreoviridin in rice in Brazil. Food Addit Contam. Part A. Chem. Anal. Control. Expo. Risk Assess. 29(4): 694-703.

Andersson, M. A., Nikulin, M., Koljalg, U., Andersson, M. C., Rainey, F., Reijula, K., Hintikka, E. L. and Salkinoja-Salonen, M. 1997. Bacteria, molds, and toxins in water-damaged building materials. Appl. Environ. Microbiol. 63(2): 387-393.

Andersson, M. A., Mikkola, R., Helin, J., Andersson, M. C. and Salkinoja-Salonen, M. 1998a. A novel sensitive bioassay for detection of *Bacillus cereus* emetic toxin and related depsipeptide ionophores. Appl. Environ. Microbiol. 64(4): 1338-1343.

Andersson, M. A., Mikkola, R., Kroppenstedt, R. M., Rainey, F. A., Peltola, J., Helin, J., Sivonen, K. and Salkinoja-Salonen, M. S. 1998b. The mitochondrial toxin produced by *Streptomyces griseus* strains isolated from an indoor environment is valinomycin. Appl. Environ. Microbiol. 64(12): 4767-4773.

Andersson, M. A., Weiss, N., Rainey, F. and Salkinoja-Salonen, M. S. 1999. Dust-borne bacteria in animal sheds, schools and children's day care centres. J. Appl. Microbiol. 86(4): 622-634.

Andersson, M. A., Jääskeläinen, E. L., Teplova, V. V., Veijalainen, P., Apetroaie, C., Hoornstra, D., Kroppenstedt, R. M. and Salkinoja-Salonen, M. S. 2005. Indoor bacilli and streptomycetes produce substances toxic to mammalian cells. In: Johanning, E. (ed.). Bioaerosols, fungi, bacteria, mycotoxins and human health: patho-physiology, clinical effects, exposure assessment, prevention and control in indoor environments and work. Fungal Research Group Foundation, Albany, NY. p. 292-299.

Andersson, M. A. and Salkinoja-Salonen, M. 2005. New toxic effects of cereulide, the emetic toxin of *Bacillus cereus*, recorded in boar sperm and Caco2 cells. Toxicol. Lett. 158(S1): P3-17.

Andersson, M. A., Hakulinen, P., Honkalampi-Hämälainen, U., Hoornstra, D., Lhuguenot, J. C., Mäki-Paakkanen, J., Savolainen, M., Severin, I., Stammati, A. L., Turco, L., Weber, A., von Wright, A., Zucco, F. and Salkinoja-Salonen, M. 2007. Toxicological profile of cereulide, the *Bacillus cereus* emetic toxin, in functional assays with human, animal and bacterial cells. Toxicon 49(3): 351-367.

Andersson, M. A., Mikkola, R., Raulio, M., Kredics, L., Maijala, P. and Salkinoja-Salonen, M. S. 2009. Acrebol, a novel toxic peptaibol produced by an *Acremonium exuviarum* indoor isolate. J. Appl. Microbiol. 106(3): 909-923.

Anon. 2010. Section 12.2: Probes for Mitochondria. In: The Molecular Probes® Handbook - A Guide to Fluorescent Probes and Labeling Technologies. Thermo Fisher Scientific Inc., Waltham, MA, USA. p. 503-516.

Anon. 2011. Ohje siivoukseen ja irtaimiston puhdistukseen kosteus- ja homevauriokorjausten jälkeen. Moisture and mould programme. Ministry of the Environment, Helsinki, Finland. 10 pp. [in Finnish]

Apetroaie, C., Andersson, M. A., Spröer, C., Tsitko, I., Shaheen, R., Jääskeläinen, E. L., Wijnands, L. M., Heikkilä, R. and Salkinoja-Salonen, M. S. 2005. Cereulide-producing strains of *Bacillus cereus* show diversity. Arch. Microbiol. 184(3): 141-151.

Apetroaie-Constantin, C., Mikkola, R., Andersson, M. A., Teplova, V., Suominen, I., Johansson, T. and Salkinoja-Salonen, M. 2009. *Bacillus subtilis* and *B. mojavensis* strains connected to food poisoning produce the heat stable toxin amylosin. J. Appl. Microbiol. 106(6): 1976-1985.

Arduengo, M. 2012. Differentiating Mitochondrial Toxicity from Other Types of Mechanistic Toxicity. http://fi.promega.com/resources/pubhub/differentiating-mitochondrial-toxicity-from-other-types-of-mechanistic-toxicity/. [Accessed 1.6.2015]

Balcke, G. U., Kolle, S. N., Kamp, H., Bethan, B., Looser, R., Wagner, S., Landsiedel, R. and van Ravenzwaay, B. 2011. Linking energy metabolism to dysfunctions in mitochondrial respiration - a metabolomics *in vitro* approach. Toxicol. Lett. 203(3): 200-209.

Berdy, J. 2012. Thoughts and facts about antibiotics: where we are now and where we are heading. J. Antibiot. (Tokyo) 65(8): 385-395.

Birch-Machin, M. A. 2008. Assessment of Mitochodrial Respiratory Complex Function *In Vitro* and *In Vivo*. In: Dykens, J. A. and Will, Y. (eds.). Drug-induced Mitochondrial Dysfunction. John Wiley & Sons, Hoboken, N.J., USA. p. 383-395.

Blais, L. A., ApSimon, J. W., Blackwell, B. A., Greenhalgh, R. and Miller, J. D. 1992. Isolation and characterization of enniatins from *Fusarium avenaceum* DAOM 196490. Can. J. Chem. 70(5): 1281-1287.

Blumer, C. and Haas, D. 2000. Mechanism, regulation, and ecological role of bacterial cyanide biosynthesis. Arch. Microbiol. 173(3): 170-177.

Bodin, J., Stene, L. C. and Nygaard, U. C. 2015. Can exposure to environmental chemicals increase the risk of diabetes type 1 development? Biomed. Res. Int. 2015: 208947.

Bose, S., Segovia, J. A., Somarajan, S. R., Chang, T. H., Kannan, T. R. and Baseman, J. B. 2014. ADP-ribosylation of NLRP3 by *Mycoplasma pneumoniae* CARDS toxin regulates inflammasome activity. MBio 5(6): e02186-14.

Boukamp, P., Petrussevska, R. T., Breitkreutz, D., Hornung, J., Markham, A. and Fusenig, N. E. 1988. Normal keratinization in a spontaneously immortalized aneuploid human keratinocyte cell line. J. Cell Biol. 106(3): 761-771.

Brand, M. D. and Nicholls, D. G. 2011. Assessing mitochondrial dysfunction in cells. Biochem. J. 435(2): 297-312.

Brockmann, H. and Schmidt-Kastner, G. 1955. Valinomycin I, XXVII. Mitteil. über Antibiotica aus Actinomyceten. Chem. Ber. 88(1): 57-61.

Busse, H. J., Denner, E. B. M., Buczolits, S., Salkinoja-Salonen, M., Bennasar, A. and Kämpfer, P. 2003. *Sphingomonas aurantiaca* sp. nov., *Sphingomonas aerolata* sp. nov. and *Sphingomonas faeni* sp. nov., air- and dustborne and Antarctic, orange-pigmented, psychrotolerant bacteria, and emended description of the genus *Sphingomonas*. Int. J. Syst. Evol. Microbiol. 53: 1253-1260.

Campos, A. and Vasconcelos, V. 2010. Molecular mechanisms of microcystin toxicity in animal cells. Int. J. Mol. Sci. 11(1): 268-287.

Canivell, S. and Gomis, R. 2014. Diagnosis and classification of autoimmune diabetes mellitus. Autoimmun. Rev. 13(4-5): 403-407.

Cantley, J., Grey, S. T., Maxwell, P. H. and Withers, D. J. 2010. The hypoxia response pathway and beta-cell function. Diabetes Obes. Metab. 12(S2): 159-167.

Carlin, F., Guinebretiere, M. H., Choma, C., Pasqualini, R., Braconnier, A. and Nguyen-The, C. 2000. Spore-forming bacteria in commercial cooked, pasteurised and chilled vegetable purees. Food Microbiol. 17(2): 153-165.

Carlin, F., Fricker, M., Pielaat, A., Heisterkamp, S., Shaheen, R., Salkinoja Salonen, M., Svensson, B., Nguyen-the, C. and Ehling-Schulz, M. 2006. Emetic toxin-producing strains of *Bacillus cereus* show distinct characteristics within the *Bacillus cereus* group. Int. J. Food Microbiol. 109(1–2): 132-138.

Chen, H. and Yan, X. 2005. Antioxidant activities of agaro-oligosaccharides with different degrees of polymerization in cell-based system. Biochim. Biophys. Acta 1722(1): 103-111.

Choi, E. M. and Lee, Y. S. 2011. Mitochondrial defects and cytotoxicity by antimycin A on cultured osteoblastic MC3T3-E1 cells. Food Chem. Toxicol. 49(9): 2459-2463.

Christensen, M. E., Jansen, E. S., Sanchez, W. and Waterhouse, N. J. 2013. Flow cytometry based assays for the measurement of apoptosis-associated mitochondrial membrane depolarisation and cytochrome *c* release. Methods 61(2): 138-145.

Claassens, O. E., Wehr, J. B. and Harrison, K. L. 2000. Optimizing sensitivity of the human sperm motility assay for embryo toxicity testing. Hum. Reprod. 15(7): 1586-1591.

Clark, I. A. 2007. The advent of the cytokine storm. Immunol. Cell Biol. 85(4): 271-273.

Coorevits, A., De Jonghe, V., Vandroemme, J., Reekmans, R., Heyrman, J., Messens, W., De Vos, P. and Heyndrickx, M. 2008. Comparative analysis of the diversity of aerobic spore-forming bacteria in raw milk from organic and conventional dairy farms. Syst. Appl. Microbiol. 31(2): 126-140.

Coppey, J., Dureiux, C. and Coppey-Moisan, M. 1999. Electrochemical Gradient and Mitochondrial DNA in Living Cells. In: Lestienne, P. (ed.). Mitochondrial diseases: models and methods. Springer-Verlag, Berlin, Germany. p. 185-196.

Cordoba-Rodriguez, R., Fang, H., Lankford, C. S. and Frucht, D. M. 2004. Anthrax lethal toxin rapidly activates caspase-1/ICE and induces extracellular release of interleukin (IL)-1beta and IL-18. J. Biol. Chem. 279(20): 20563-20566.

Cross, R. L. and Kohlbrenner, W. E. 1978. The mode of inhibition of oxidative phosphorylation by efrapeptin (A23871). Evidence for an alternating site mechanism for ATP synthesis. J. Biol. Chem. 253(14): 4865-4873.

Curtis, L., Lieberman, A., Stark, M., Rea, W. and Vetter, M. 2004. Adverse Health Effects of Indoor Molds. J. Nutr. Environ. Med. 14(3): 261-274.

Daley, D. 2014. The evolution of the hygiene hypothesis: the role of early-life exposures to viruses and microbes and their relationship to asthma and allergic diseases. Curr. Opin. Allergy Clin. Immunol. 14(5): 390-396.

de Baar, M. P. and de Ronde, A. 2008. Mitochondrial Toxicity of Antiviral Drugs: A Challenge to Accurate Diagnosis. In: Dykens, J. A. and Will, Y. (eds.). Drug-induced Mitochondrial Dysfunction. John Wiley & Sons, Hoboken, N.J., USA. p. 473-491.

De Jonghe, V., Coorevits, A., De Block, J., Van Coillie, E., Grijspeerdt, K., Herman, L., De Vos, P. and Heyndrickx, M. 2010. Toxinogenic and spoilage potential of aerobic spore-formers isolated from raw milk. Int. J. Food Microbiol. 136(3): 318-325.

De Nardo, D., De Nardo, C. M. and Latz, E. 2014. New Insights into Mechanisms Controlling the NLRP3 Inflammasome and Its Role in Lung Disease. Am. J. Pathol. 184(1): 42-54.

Delbrassinne, L., Andjelkovic, M., Rajkovic, A., Bottledoorn, N., Mahillon, J. and Van Loco, J. 2011. Follow-up of the *Bacillus cereus* emetic toxin production in penne pasta under household conditions using liquid chromatography coupled with mass spectrometry. Food Microbiol. 28(5): 1105-1109.

Dierick, K., Van Coillie, E., Swiecicka, I., Meyfroidt, G., Devlieger, H., Meulemans, A., Hoedemaekers, G., Fourie, L., Heyndrickx, M. and Mahillon, J. 2005. Fatal family outbreak of *Bacillus cereus*-associated food poisoning. J. Clin. Microbiol. 43(8): 4277-4279.

DiMauro, S., Hirano, M. and Schon, E. A. (eds.). 2006. Mitochondrial medicine. Informa Healthcare, London, UK. 348 pp.

Dinarello, C. A. 2009. Immunological and inflammatory functions of the interleukin-1 family. Annu. Rev. Immunol. 27: 519-550.

Doyle, M. P. and Buchanan, R. 2013. Food microbiology: fundamentals and frontiers (4^{th} ed.). ASM Press, Washington, D.C., USA. p. 225-573, 597-649,737-822.

Dykens, J. A. and Will, Y. 2007. The significance of mitochondrial toxicity testing in drug development. Drug Discov. Today 12(17-18): 777-785.

ECHA. 2014. European Chemicals Agency. 2014. Factsheet - Role of animal testing in ensuring the safe use of chemical substances. ECHA-12-FS-08-EN. http://echa.europa.eu/documents/10162/13630/reach_factsheet_animal_testing_en.pdf.

Echard, G. 1974. Chromosomal banding patterns and karyotype evolution in three pig kidney cell strains (PK15, F and RP). Chromosoma 45(2): 133-149.

EFSA (European Food Safety Authority) and ECDC (European Centre for Disease Prevention and Control). 2012. The European Union Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents and Food-borne Outbreaks in 2010. EFSA Journal 10(3): 2597, 442 pp.

EFSA (European Food Safety Authority) and ECDC (European Centre for Disease Prevention and Control). 2015. The European Union Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents and Food-borne Outbreaks in 2014. EFSA Journal 13(12): 4329, 191 pp.

Egro, F. M. 2013. Why is type 1 diabetes increasing? J. Mol. Endocrinol. 51(1): R1-13.

Ehling-Schulz, M., Svensson, B., Guinebretiere, M. H., Lindback, T., Andersson, M., Schulz, A., Fricker, M., Christiansson, A., Granum, P. E., Martlbauer, E., Nguyen-The, C., Salkinoja-Salonen, M. and Scherer, S. 2005. Emetic toxin formation of *Bacillus cereus* is restricted to a single evolutionary lineage of closely related strains. Microbiology 151(Pt 1): 183-197.

Eirin, A., Lerman, A. and Lerman, L. O. 2014. Mitochondrial injury and dysfunction in hypertension-induced cardiac damage. Eur. Heart J. 35(46): 3258-3266.

Ekman, J. V., Kruglov, A., Andersson, M. A., Mikkola, R., Raulio, M. and Salkinoja-Salonen, M. 2012. Cereulide produced by *Bacillus cereus* increases the fitness of the producer organism in low-potassium environments. Microbiology 158(Pt 4): 1106-1116.

Elliott, E. I. and Sutterwala, F. S. 2015. Initiation and perpetuation of NLRP3 inflammasome activation and assembly. Immunol. Rev. 265(1): 35-52.

EVIRA. 2015. Finnish Food Safety Authority official web pages. http://www.evira.fi/. [Accessed 24.8.2015]

Fangio, M. F., Roura, S. I. and Fritz, R. 2010. Isolation and identification of *Bacillus* spp. and related genera from different starchy foods. J. Food Sci. 75(4): M218-21.

Fantin, V. R. and Leder, P. 2006. Mitochondriotoxic compounds for cancer therapy. Oncogene 25(34): 4787-4797.

Ferrick, D., Wu, M., Swift, A. and Neilson, A. 2008. Mitochondrial Dysfunction Assessed Quantitatively in Real Time by Measuring the Extracellular Flux of Oxygen and Protons. In: Dykens, J. A. and Will, Y. (eds.). Drug-induced Mitochondrial Dysfunction. John Wiley & Sons, Hoboken, N.J., USA. p. 373-382.

Fisk, W. J., Lei-Gomez, Q. and Mendell, M. J. 2007. Meta-analyses of the associations of respiratory health effects with dampness and mold in homes. Indoor Air 17(4): 284-296.

Florian, M., Yan, J., Ulhaq, S., Coughlan, M., Laziyan, M., Willmore, W. and Jin, X. 2013. Northern contaminant mixtures induced morphological and functional changes in human coronary artery endothelial cells under culture conditions typifying high fat/sugar diet and ethanol exposure. Toxicology 313(2-3): 103-112.

Francis, K., Smitherman, C., Nishino, S. F., Spain, J. C. and Gadda, G. 2013. The biochemistry of the metabolic poison propionate 3-nitronate and its conjugate acid, 3-nitropropionate. IUBMB Life 65(9): 759-768.

Frankland, G. C. and Frankland, P. F. 1887. Studies on Some New Micro-Organisms Obtained from Air. Philosophical Transactions of the Royal Society of London B: Biological Sciences 178: 257-287.

Freyre-Fonseca, V., Delgado-Buenrostro, N. L., Gutiérrez-Cirlos, E. B., Calderón-Torres, C. M., Cabellos-Avelar, T., Sánchez-Pérez, Y., Pinzón, E., Torres, I., Molina-Jijón, E., Zazueta, C., Pedraza-Chaverri, J., García-Cuéllar, C. M. and Chirino, Y. I. 2011. Titanium dioxide nanoparticles impair lung mitochondrial function. Toxicol. Lett. 202(2): 111-119.

Friedrich, T., van Heek, P., Leif, H., Ohnishi, T., Forche, E., Kunze, B., Jansen, R., Trowitzsch-Kienast, W., Hofle, G. and Reichenbach, H. 1994. Two binding sites of inhibitors in NADH: ubiquinone oxidoreductase (complex I). Relationship of one site with the ubiquinone-binding site of bacterial glucose:ubiquinone oxidoreductase. Eur. J. Biochem. 219(1-2): 691-698.

Fromm, H. I. and Boor, K. J. 2004. Characterization of pasteurized fluid milk shelf-life attributes. J. Food Sci. 69(8): M207-M214.

Fünfhaus, A., Poppinga, L. and Genersch, E. 2013. Identification and characterization of two novel toxins expressed by the lethal honey bee pathogen *Paenibacillus larvae*, the causative agent of American foulbrood. Environ. Microbiol. 15(11): 2951-2965.

Galluzzi, L., Kepp, O., Trojel-Hansen, C. and Kroemer, G. 2012. Mitochondrial control of cellular life, stress, and death. Circ. Res. 111(9): 1198-1207.

Gammelsrud, A., Solhaug, A., Dendelé, B., Sandberg, W. J., Ivanova, L., Kocbach Bølling, A., Lagadic-Gossmann, D., Refsnes, M., Becher, R., Eriksen, G. and Holme, J. A. 2012. Enniatin B-induced cell death and inflammatory responses in RAW 267.4 murine macrophages. Toxicol. Appl. Pharmacol. 261(1): 74-87.

Gani, O. A. and Engh, R. A. 2010. Protein kinase inhibition of clinically important staurosporine analogues. Nat. Prod. Rep. 27(4): 489-498.

Gerst, M. M., Huang, E., Zhang, L. and Yousef, A. E. 2015. Development of a New Paenibacillin-Producing Strain and Testing its Usability in Improving Food Safety. J. Food Sci. 80(7): M1538-43.

Girardin, H., Albagnac, C., Dargaignaratz, C., Nguyen-The, C. and Carlin, F. 2002. Antimicrobial activity of foodborne *Paenibacillus* and *Bacillus* spp. against *Clostridium botulinum*. J. Food Prot. 65(5): 806-813.

Global Burden of Disease Study 2013 Collaborators. 2015. Global, regional, and national incidence, prevalence, and years lived with disability for 301 acute and chronic diseases and injuries in 188 countries, 1990-2013: a systematic analysis for the Global Burden of Disease Study 2013. Lancet 386(9995): 743-800.

Gnaiger, E. 2008. Polarographic Oxygen Sensors, the Oxygraph, and High-Resolution Respirometry to Assess Mitochondrial Function. In: Dykens, J. A. and Will, Y. (eds.). Drug-induced Mitochondrial Dysfunction. John Wiley & Sons, Hoboken, N.J., USA. p. 327-352.

Guinebretiere, M. H., Berge, O., Normand, P., Morris, C., Carlin, F. and Nguyen-The, C. 2001. Identification of bacteria in pasteurized zucchini purees stored at different temperatures and comparison with those found in other pasteurized vegetable purees. Appl. Environ. Microbiol. 67(10): 4520-4530.

Gulden, M., Morchel, S. and Seibert, H. 2001. Factors influencing nominal effective concentrations of chemical compounds *in vitro*: cell concentration. Toxicol. in Vitro 15(3): 233-243.

Gupta, R., Ghosh, S., Monks, B., DeOliveira, R. B., Tzeng, T. C., Kalantari, P., Nandy, A., Bhattacharjee, B., Chan, J., Ferreira, F., Rathinam, V., Sharma, S., Lien, E., Silverman, N., Fitzgerald, K., Firon, A., Trieu-Cuot, P., Henneke, P. and Golenbock, D. T. 2014. RNA and beta-hemolysin of group B *Streptococcus* induce interleukin-1beta (IL-1β) by activating NLRP3 inflammasomes in mouse macrophages. J. Biol. Chem. 289(20): 13701-13705.

Gurung, P., Lukens, J. R. and Kanneganti, T. D. 2015. Mitochondria: diversity in the regulation of the NLRP3 inflammasome. Trends Mol. Med. 21(3): 193-201.

Gäumann, E., Roth, S., Ettlinger, L., Plattner, P. A. and Nager, U. 1947. Enniatin, ein neues, gegen Mykobakterien wirksames Antibiotikum. Experientia 3(5): 202-203.

Hallaksela, A., Väisänen, O. and Salkinoja-Salonen, M. 1991. Identification of *Bacillus* Species Isolated from *Picea abies* by Physiological Tests, Phage Typing and Fatty Acid Analysis. Scand. J. For. Res. 6(1-4): 365-377.

Han, S., Auger, C., Castonguay, Z., Appanna, V. P., Thomas, S. C. and Appanna, V. D. 2013. The unravelling of metabolic dysfunctions linked to metal-associated diseases by blue native polyacrylamide gel electrophoresis. Anal. Bioanal Chem. 405(6): 1821-1831.

Hawthorne, S. K., Goodarzi, G., Bagarova, J., Gallant, K. E., Busanelli, R. R., Olend, W. J. and Kleene, K. C. 2006. Comparative genomics of the sperm mitochondria-associated cysteine-rich protein gene. Genomics 87(3): 382-391.

Heseltine, E., Rosen, J. and World Health Organization. Regional Office for Europe. 2009. WHO guidelines for indoor air quality: dampness and mould. World Health Organization, Regional Office for Europe, Copenhagen, Denmark. 228 pp.

Hettiarachchi, K. D., Zimmet, P. Z. and Myers, M. A. 2006. The effects of repeated exposure to sub-toxic doses of plecomacrolide antibiotics on the endocrine pancreas. Food Chem. Toxicol. 44(12): 1966-1977.

Hirvonen, M. R., Huttunen, K. and Roponen, M. 2005. Bacterial strains from moldy buildings are highly potent inducers of inflammatory and cytotoxic effects. Indoor Air 15(S9): 65-70.

Hodgson, M. 1992. Field studies on the sick building syndrome. Ann. N. Y. Acad. Sci. 641: 21-36.

Holzinger, D., Gieldon, L., Mysore, V., Nippe, N., Taxman, D. J., Duncan, J. A., Broglie, P. M., Marketon, K., Austermann, J., Vogl, T., Foell, D., Niemann, S., Peters, G., Roth, J. and Loffler, B. 2012. *Staphylococcus aureus* Panton-Valentine leukocidin induces an inflammatory response in human phagocytes via the NLRP3 inflammasome. J. Leukoc. Biol. 92(5): 1069-1081.

Hoornstra, D., Andersson, M. A., Mikkola, R. and Salkinoja-Salonen, M. S. 2003. A new method for *in vitro* detection of microbially produced mitochondrial toxins. Toxicol. in Vitro 17(5-6): 745-751.

Hoornstra, D., Andersson, M. A., Teplova, V. V., Mikkola, R., Uotila, L. M., Andersson, L. C., Roivainen, M., Gahmberg, C. G. and Salkinoja-Salonen, M. S. 2013. Potato crop as a source of emetic *Bacillus cereus* and cereulide-induced mammalian cell toxicity. Appl. Environ. Microbiol. 79(12): 3534-3543.

Hoschele, D. 2006. Cell culture models for the investigation of NRTI-induced mitochondrial toxicity. Relevance for the prediction of clinical toxicity. Toxicol. in Vitro 20(5): 535-546.

Hosseinian, N., Cho, Y., Lockey, R. F. and Kolliputi, N. 2015. The role of the NLRP3 inflammasome in pulmonary diseases. Ther. Adv. Respir. Dis. 9(4): 188-197.

Huck, J. R., Sonnen, M. and Boor, K. J. 2008. Tracking heat-resistant, cold-thriving fluid milk spoilage bacteria from farm to packaged product. J. Dairy Sci. 91(3): 1218-1228.

Husman, T. 2002. Kosteusvauriotaloissa asuneiden perheiden pitkäaikaiset terveyshaitat ja asumisterveysongelmista aiheutuneet kustannukset. Kansanterveyslaitoksen julkaisuja B5/2002. National Public Health Institute of Finland, Helsinki, Finland. 33 pp. [in Finnish]

Hynes, J., Hill, R. and Papkovsky, D. B. 2006. The use of a fluorescence-based oxygen uptake assay in the analysis of cytotoxicity. Toxicol. in Vitro 20(5): 785-792.

Hynes, J., O'Riordan, T. C. and Papkovsky, D. B. 2008. Use of Oxygen-Sensitive Fluorescent Probes for the Assessment of Mitochondrial Function. In: Dykens, J. A. and Will, Y. (eds.). Drug-induced Mitochondrial Dysfunction. John Wiley & Sons, Hoboken, N.J., USA. p. 353-371.

Hynes, J., Nadanaciva, S., Swiss, R., Carey, C., Kirwan, S. and Will, Y. 2013. A high-throughput dual parameter assay for assessing drug-induced mitochondrial dysfunction provides additional predictivity over two established mitochondrial toxicity assays. Toxicol. in Vitro 27(2): 560-569.

Häggblom, M. M., Apetroaie, C., Andersson, M. A. and Salkinoja-Salonen, M. S. 2002. Quantitative analysis of cereulide, the emetic toxin of *Bacillus cereus*, produced under various conditions. Appl. Environ. Microbiol. 68(5): 2479-2483.

Ichikawa, K., Gakumazawa, M., Inaba, A., Shiga, K., Takeshita, S., Mori, M. and Kikuchi, N. 2010. Acute encephalopathy of *Bacillus cereus* mimicking Reye syndrome. Brain Dev. 32(8): 688-690.

IOM. 2004. Institute of Medicine (U.S.). Committee on Damp Indoor Spaces and Health. Damp indoor spaces and health. National Academies Press, Washington, D.C., USA. 355 pp.

IOM. 2011. Institute of Medicine (U.S.). Committee on the Effect of Climate Change on Indoor Air Quality and Public Health: Climate change, the indoor environment, and health. National Academies Press, Washington, D.C., USA. 272 pp.

Ivy, R. A., Ranieri, M. L., Martin, N. H., den Bakker, H. C., Xavier, B. M., Wiedmann, M. and Boor, K. J. 2012. Identification and characterization of psychrotolerant sporeformers associated with fluid milk production and processing. Appl. Environ. Microbiol. 78(6): 1853-1864.

Jabaut, J., Ather, J. L., Taracanova, A., Poynter, M. E. and Ckless, K. 2013. Mitochondria-targeted drugs enhance Nlrp3 inflammasome-dependent IL-1beta secretion in association with alterations in cellular redox and energy status. Free Radic. Biol. Med. 60: 233-245.

Jarchum, I. and Pamer, E. G. 2011. Regulation of innate and adaptive immunity by the commensal microbiota. Curr. Opin. Immunol. 23(3): 353-360.

Jeger, V., Brandt, S., Porta, F., Jakob, S. M., Takala, J. and Djafarzadeh, S. 2015. Dose response of endotoxin on hepatocyte and muscle mitochondrial respiration *in vitro*. Biomed. Res. Int. 2015: 353074.

Jestoi, M., Rokka, M., Yli-Mattila, T., Parikka, P., Rizzo, A. and Peltonen, K. 2004a. Presence and concentrations of the *Fusarium*-related mycotoxins beauvericin, enniatins and moniliformin in Finnish grain samples. Food Addit. Contam. 21(8): 794-802.

Jestoi, M., Somma, M. C., Kouva, M., Veijalainen, P., Rizzo, A., Ritieni, A. and Peltonen, K. 2004b. Levels of mycotoxins and sample cytotoxicity of selected organic and conventional grain-based products purchased from Finnish and Italian markets. Mol. Nutr. Food Res. 48(4): 299-307.

Jestoi, M. 2008. Emerging fusarium-mycotoxins fusaproliferin, beauvericin, enniatins, and moniliformin: a review. Crit. Rev. Food Sci. Nutr. 48(1): 21-49.

Julia, V., Macia, L. and Dombrowicz, D. 2015. The impact of diet on asthma and allergic diseases. Nat. Rev. Immunol. 15(5): 308-322.

Jääskeläinen, E. L., Teplova, V., Andersson, M. A., Andersson, L. C., Tammela, P., Andersson, M. C., Pirhonen, T. I., Saris, N. E., Vuorela, P. and Salkinoja-Salonen, M. S. 2003. *In vitro* assay for human toxicity of cereulide, the emetic mitochondrial toxin produced by food poisoning *Bacillus cereus*. Toxicol. in Vitro 17(5-6): 737-744.

Kamyar, M., Rawnduzi, P., Studenik, C. R., Kouri, K. and Lemmens-Gruber, R. 2004. Investigation of the electrophysiological properties of enniatins. Arch. Biochem. Biophys. 429(2): 215-223.

Kankkunen, P., Rintahaka, J., Aalto, A., Leino, M., Majuri, M. L., Alenius, H., Wolff, H. and Matikainen, S. 2009. Trichothecene mycotoxins activate inflammatory response in human macrophages. J. Immunol. 182(10): 6418-6425.

Karmakar, M., Katsnelson, M., Malak, H. A., Greene, N. G., Howell, S. J., Hise, A. G., Camilli, A., Kadioglu, A., Dubyak, G. R. and Pearlman, E. 2015. Neutrophil IL-1beta processing induced by pneumolysin is mediated by the NLRP3/ASC inflammasome and caspase-1 activation and is dependent on K+ efflux. J. Immunol. 194(4): 1763-1775.

Katou, Y., Endo, N., Suzuki, T., Yu, J., Kikuchi, H., Oshima, Y. and Homma, Y. 2014. Metarhizin A suppresses cell proliferation by inhibiting cytochrome c oxidase activity. Life Sci. 103(1): 1-7.

Kebaier, C., Chamberland, R. R., Allen, I. C., Gao, X., Broglie, P. M., Hall, J. D., Jania, C., Doerschuk, C. M., Tilley, S. L. and Duncan, J. A. 2012. *Staphylococcus aureus* alpha-hemolysin mediates virulence in a murine model of severe pneumonia through activation of the NLRP3 inflammasome. J. Infect. Dis. 205(5): 807-817.

Kilkkinen, A., Virtanen, S. M., Klaukka, T., Kenward, M. G., Salkinoja-Salonen, M., Gissler, M., Kaila, M. and Reunanen, A. 2006. Use of antimicrobials and risk of type 1 diabetes in a population-based mother-child cohort. Diabetologia 49(1): 66-70.

Ko, K. S., Kim, Y. S., Lee, M. Y., Shin, S. Y., Jung, D. S., Peck, K. R. and Song, J. H. 2008. *Paenibacillus konsidensis* sp. nov., isolated from a patient. Int. J. Syst. Evol. Microbiol. 58(Pt 9): 2164-2168.

Kondrashova, A., Seiskari, T., Ilonen, J., Knip, M. and Hyoty, H. 2013. The 'Hygiene hypothesis' and the sharp gradient in the incidence of autoimmune and allergic diseases between Russian Karelia and Finland. APMIS 121(6): 478-493.

Korpi, A., Pasanen, A.-L., Pasanen, P. and Kalliokoski, P. 1997. Microbial growth and metabolism in house dust. Int. Biodeterior. Biodegrad. 40(1): 19-27.

Kotiaho, M., Aittamaa, M., Andersson, M. A., Mikkola, R., Valkonen, J. P. and Salkinoja-Salonen, M. 2008. Antimycin A-producing nonphytopathogenic *Streptomyces turgidiscabies* from potato. J. Appl. Microbiol. 104(5): 1332-1340.

Krasnoff, S. B. and Gupta, S. 1991. Identification and directed biosynthesis of efrapeptins in the fungus *Tolypocladium geodes* gams (Deuteromycotina: Hyphomycetes). J. Chem. Ecol. 17(10): 1953-1962.

Krska, D., Ravulapalli, R., Fieldhouse, R. J., Lugo, M. R. and Merrill, A. R. 2015. C3larvin toxin, an ADP-ribosyltransferase from *Paenibacillus larvae*. J. Biol. Chem. 290(3): 1639-1653.

Kruglov, A. G., Andersson, M. A., Mikkola, R., Roivainen, M., Kredics, L., Saris, N. E. and Salkinoja-Salonen, M. S. 2009. Novel mycotoxin from *Acremonium exuviarum* is a powerful inhibitor of the mitochondrial respiratory chain complex III. Chem. Res. Toxicol. 22(3): 565-573.

Kunze, B., Kemmer, T., Höfle, G. and Reichenbach, H. 1984. Stigmatellin, a new antibiotic from *Stigmatella aurantiaca* (Myxobacterales). I. Production, physico-chemical and biological properties. J. Antibiot. (Tokyo) 37(5): 454-461.

Kämpfer, P., Andersson, M. A., Rainey, F. A., Kroppenstedt, R. M. and Salkinoja-Salonen, M. 1999. *Williamsia muralis* gen. nov., sp. nov., isolated from the indoor environment of a children's day care centre. Int. J. Syst. Bacteriol. 49(2): 681-687.

Kärki, J. and Öhman, H. 2007. Homevaurioiden korjausopas. University of Kuopio, Kuopio, Finland. 70 pp. [in Finnish]

Labbe, G., Pessayre, D. and Fromenty, B. 2008. Drug-induced liver injury through mitochondrial dysfunction: mechanisms and detection during preclinical safety studies. Fundam. Clin. Pharmacol. 22(4): 335-353.

Ladeuze, S., Lentz, N., Delbrassinne, L., Hu, X. and Mahillon, J. 2011. Antifungal activity displayed by cereulide, the emetic toxin produced by *Bacillus cereus*. Appl. Environ. Microbiol. 77(7): 2555-2558.

Lee, S. Y., Cherla, R. P., Caliskan, I. and Tesh, V. L. 2005. Shiga toxin 1 induces apoptosis in the human myelogenous leukemia cell line THP-1 by a caspase-8-dependent, tumor necrosis factor receptor-independent mechanism. Infect. Immun. 73(8): 5115-5126.

Leemans, J. C., Cassel, S. L. and Sutterwala, F. S. 2011. Sensing damage by the NLRP3 inflammasome. Immunol. Rev. 243(1): 152-162.

Leist, M., Single, B., Castoldi, A. F., Kuhnle, S. and Nicotera, P. 1997. Intracellular adenosine triphosphate (ATP) concentration: a switch in the decision between apoptosis and necrosis. J. Exp. Med. 185(8): 1481-1486.

Lemasters, J. J. 2014. Variants of mitochondrial autophagy: Types 1 and 2 mitophagy and micromitophagy (Type 3). Redox Biol. 2: 749-754.

Lennon, R. E., Hunn, J. B. and Schnick, R. A. 1971. Chapter 5: Chemicals employed as fish toxicants. In: Reclamation of Ponds, Lakes, and Streams with Fish Toxicants: A Review. Food and Agriculture Organization (FAO) of the United Nations, Rome, Italy.

Lierman, S., De Sutter, P., Dhont, M. and Van der Elst, J. 2007. Double-quality control reveals high-level toxicity in gloves used for operator protection in assisted reproductive technology. Fertil. Steril. 88(S4): 1266-1272.

Linnett, P. E. and Beechey, R. B. 1979. Inhibitors of the ATP synthethase system. Methods Enzymol. 55: 472-518.

Liu, C. and Hermann, T. E. 1978. Characterization of ionomycin as a calcium ionophore. J. Biol. Chem. 253(17): 5892-5894.

Lunt, S. Y. and Vander Heiden, M. G. 2011. Aerobic glycolysis: meeting the metabolic requirements of cell proliferation. Annu. Rev. Cell Dev. Biol. 27: 441-464.

Luu-Thi, H., Khadka, D. B. and Michiels, C. W. 2014. Thermal inactivation parameters of spores from different phylogenetic groups of *Bacillus cereus*. Int. J. Food Microbiol. 189: 183-188.

Maechler, P., Carobbio, S. and Rubi, B. 2006. In beta-cells, mitochondria integrate and generate metabolic signals controlling insulin secretion. Int. J. Biochem. Cell Biol. 38(5-6): 696-709.

Mahler, H., Pasi, A., Kramer, J. M., Schulte, P., Scoging, A. C., Bär, W. and Krähenbühl, S. 1997. Fulminant liver failure in association with the emetic toxin of *Bacillus cereus*. N. Engl. J. Med. 336(16): 1142-1148.

Mailloux, R., Fu, A., Florian, M., Petrov, I., Chen, Q., Coughlan, M. C., Laziyan, M., Yan, J., Caldwell, D., Patry, D., Lalande, M., Wang, G. S., Willmore, W. and Jin, X. 2015. A Northern contaminant mixture impairs pancreas function in obese and lean JCR rats and inhibits insulin secretion in MIN6 cells. Toxicology 334: 81-93.

Mailloux, R. J., Florian, M., Chen, Q., Yan, J., Petrov, I., Coughlan, M. C., Laziyan, M., Caldwell, D., Lalande, M., Patry, D., Gagnon, C., Sarafin, K., Truong, J., Chan, H. M., Ratnayake, N., Li, N., Willmore, W. G. and Jin, X. 2014. Exposure to a northern contaminant mixture (NCM) alters hepatic energy and lipid metabolism exacerbating hepatic steatosis in obese JCR rats. PLoS One 9(9): e106832.

Mariathasan, S., Weiss, D. S., Newton, K., McBride, J., O'Rourke, K., Roose-Girma, M., Lee, W. P., Weinrauch, Y., Monack, D. M. and Dixit, V. M. 2006. Cryopyrin activates the inflammasome in response to toxins and ATP. Nature 440(7081): 228-232.

Marroquin, L. D., Hynes, J., Dykens, J. A., Jamieson, J. D. and Will, Y. 2007. Circumventing the Crabtree effect: replacing media glucose with galactose increases susceptibility of HepG2 cells to mitochondrial toxicants. Toxicol. Sci. 97(2): 539-547.

Martinon, F., Burns, K. and Tschopp, J. 2002. The inflammasome: a molecular platform triggering activation of inflammatory caspases and processing of proIL-β. Mol. Cell 10(2): 417-426.

Marusich, M. F., Murray, J., Xie, J. and Capaldi, R. A. 2009. Novel antibody-based strategies for the rapid diagnosis of mitochondrial disease and dysfunction. Int. J. Biochem. Cell Biol. 41(10): 2081-2088.

Marxen, S., Stark, T. D., Frenzel, E., Rütschle, A., Lücking, G., Pärstinger, G., Pohl, E. E., Scherer, S., Ehling-Schulz, M. and Hofmann, T. 2015a. Chemodiversity of cereulide, the emetic toxin of *Bacillus cereus*. Anal. Bioanal Chem. 407(9): 2439-2453.

Marxen, S., Stark, T. D., Rütschle, A., Lücking, G., Frenzel, E., Scherer, S., Ehling-Schulz, M. and Hofmann, T. 2015b. Multiparametric Quantitation of the *Bacillus cereus* Toxins Cereulide and Isocereulides A-G in Foods. J. Agric. Food Chem. 63(37): 8307-8313.

Masiello, S. N., Martin, N. H., Watters, R. D., Galton, D. M., Schukken, Y. H., Wiedmann, M. and Boor, K. J. 2014. Identification of dairy farm management practices associated with the presence of psychrotolerant sporeformers in bulk tank milk. J. Dairy Sci. 97(7): 4083-4096.

Mathews, C. K., Van Holde, K. E. and Ahern, K. G. 2000. Biochemistry (3rd ed.). Addison Wesley Longman, San Francisco, CA, USA. 1186 pp.

Matter, A. M., Hoot, S. B., Anderson, P. D., Neves, S. S. and Cheng, Y. Q. 2009. Valinomycin biosynthetic gene cluster in *Streptomyces*: conservation, ecology and evolution. PLoS One 4(9): e7194.

McKee, E. E. 2008. Assessing Mitochondrial Protein Synthesis in Drug Toxicity Screening. In: Dykens, J. A. and Will, Y. (eds.). Drug-induced Mitochondrial Dysfunction. John Wiley & Sons, Hoboken, N.J., USA. p. 463-472.

Mendell, M. J., Mirer, A. G., Cheung, K., Tong, M. and Douwes, J. 2011. Respiratory and allergic health effects of dampness, mold, and dampness-related agents: a review of the epidemiologic evidence. Environ. Health Perspect. 119(6): 748-756.

Messelhausser, U., Frenzel, E., Blöchinger, C., Zucker, R., Kämpf, P. and Ehling-Schulz, M. 2014. Emetic *Bacillus cereus* are more volatile than thought: recent foodborne outbreaks and prevalence studies in Bavaria (2007-2013). Biomed. Res. Int. 2014: 465603.

Meyer, J. N., Leung, M. C., Rooney, J. P., Sendoel, A., Hengartner, M. O., Kisby, G. E. and Bess, A. S. 2013. Mitochondria as a target of environmental toxicants. Toxicol. Sci. 134(1): 1-17.

Mikkola, R., Saris, N. E., Grigoriev, P. A., Andersson, M. A. and Salkinoja-Salonen, M. S. 1999. Ionophoretic properties and mitochondrial effects of cereulide - the emetic toxin of *B. cereus*. Eur. J. Biochem. 263(1): 112-117.

Mikkola, R., Andersson, M. A., Grigoriev, P., Teplova, V. V., Saris, N. E., Rainey, F. A. and Salkinoja-Salonen, M. S. 2004. *Bacillus amyloliquefaciens* strains isolated from moisture-damaged buildings produced surfactin and a substance toxic to mammalian cells. Arch. Microbiol. 181(4): 314-323.

Mikkola, R. 2006. Ph. D. thesis. Food and indoor air isolated *Bacillus* non-protein toxins: structure, physico-chemical properties and mechanism of effects on eukaryotic cells. Dissertationes bioscientiarum molecularium Universitatis Helsingiensis in Viikki 33/2006. 96 pp. + 5 annexes.

Mikkola, R., Andersson, M. A., Teplova, V., Grigoriev, P., Kuehn, T., Loss, S., Tsitko, I., Apetroaie, C., Saris, N. E., Veijalainen, P. and Salkinoja-Salonen, M. S. 2007. Amylosin from *Bacillus amyloliquefaciens*, a K^+ and Na^+ channel-forming toxic peptide containing a polyene structure. Toxicon 49(8): 1158-1171.

Mikkola, R., Andersson, M. A., Hautaniemi, M. and Salkinoja-Salonen, M. S. 2015. Toxic indole alkaloids avrainvillamide and stephacidin B produced by a biocide tolerant indoor mold *Aspergillus westerdijkiae*. Toxicon 99: 58-67.

Milane, L., Trivedi, M., Singh, A., Talekar, M. and Amiji, M. 2015. Mitochondrial biology, targets, and drug delivery. J. Control. Release 207: 40-58.

Millar, A. H., Whelan, J., Soole, K. L. and Day, D. A. 2011. Organization and regulation of mitochondrial respiration in plants. Annu. Rev. Plant. Biol. 62: 79-104.

Mitani, M., Yamanishi, T. and Miyazaki, Y. 1975. Salinomycin: a new monovalent cation ionophore. Biochem. Biophys. Res. Commun. 66(4): 1231-1236.

Modica-Napolitano, J. S. and Weissig, V. 2015. Treatment Strategies that Enhance the Efficacy and Selectivity of Mitochondria-Targeted Anticancer Agents. Int. J. Mol. Sci. 16(8): 17394-17421.

Moreno Switt, A. I., Andrus, A. D., Ranieri, M. L., Orsi, R. H., Ivy, R., den Bakker, H. C., Martin, N. H., Wiedmann, M. and Boor, K. J. 2014. Genomic comparison of sporeforming bacilli isolated from milk. BMC Genomics 15: 26-2164-15-26.

Morgan, J. B., Liu, Y., Coothankandaswamy, V., Mahdi, F., Jekabsons, M. B., Gerwick, W. H., Valeriote, F. A., Zhou, Y. D. and Nagle, D. G. 2015. Kalkitoxin inhibits angiogenesis, disrupts cellular hypoxic signaling, and blocks mitochondrial electron transport in tumor cells. Mar. Drugs 13(3): 1552-1568.

Mostafalou, S. and Abdollahi, M. 2013. Pesticides and human chronic diseases: evidences, mechanisms, and perspectives. Toxicol. Appl. Pharmacol. 268(2): 157-177.

Mudarri, D. and Fisk, W. J. 2007. Public health and economic impact of dampness and mold. Indoor Air 17(3): 226-235.

Muñoz-Planillo, R., Kuffa, P., Martínez-Colón, G., Smith, B. L., Rajendiran, T. M. and Núñez, G. 2013. K^+ efflux is the common trigger of NLRP3 inflammasome activation by bacterial toxins and particulate matter. Immunity 38(6): 1142-1153.

Myers, M. A., Hettiarachchi, K. D., Ludeman, J. P., Wilson, A. J., Wilson, C. R. and Zimmet, P. Z. 2003. Dietary microbial toxins and type 1 diabetes. Ann. N. Y. Acad. Sci. 1005: 418-422.

Nadanaciva, S. 2008. OXPHOS Complex Activity Assays and Dipstick Immunoassays for Assessment of OXPHOS Protein Levels. In: Dykens, J. A. and Will, Y. (eds.). Drug-induced Mitochondrial Dysfunction. John Wiley & Sons, Hoboken, N.J., USA. p. 397-412.

Naranjo, M., Denayer, S., Botteldoorn, N., Delbrassinne, L., Veys, J., Waegenaere, J., Sirtaine, N., Driesen, R. B., Sipido, K. R., Mahillon, J. and Dierick, K. 2011. Sudden Death of a Young Adult Associated with *Bacillus cereus* Food Poisoning. J. Clin. Microbiol. 49(12): 4379-4381.

Nasu, Y., Nosaka, Y. F., Otsuka, Y. F., Tsuruga, T. F., Nakajima, M. F., Watanabe, Y. F. and Jin, M. 2003. A case of *Paenibacillus polymyxa* bacteremia in a patient with cerebral infarction. Kansenshōgaku zasshi 77(10): 844-848.

Nelson, B. D., Walter, P. and Ernster, L. 1977. Funiculosin: an antibiotic with antimycin-like inhibitory properties. Biochim. Biophys. Acta 460(1): 157-162.

Nelson, D. M., Glawe, A. J., Labeda, D. P., Cann, I. K. and Mackie, R. I. 2009. *Paenibacillus tundrae* sp. nov. and *Paenibacillus xylanexedens* sp. nov., psychrotolerant, xylan-degrading bacteria from Alaskan tundra. Int. J. Syst. Evol. Microbiol. 59(Pt 7): 1708-1714.

Neustadt, J. and Pieczenik, S. R. 2008. Medication-induced mitochondrial damage and disease. Mol. Nutr. Food Res. 52(7): 780-788.

Nevalainen, A., Täubel, M. and Hyvärinen, A. 2015. Indoor fungi: companions and contaminants. Indoor Air 25(2): 125-156.

Newton, A. P., Cadena, S. M., Rocha, M. E., Carnieri, E. G. and Martinelli de Oliveira, M. B. 2005. Effect of triclosan (TRN) on energy-linked functions of rat liver mitochondria. Toxicol. Lett. 160(1): 49-59.

Ng, J., Hirota, S. A., Gross, O., Li, Y., Ulke-Lemee, A., Potentier, M. S., Schenck, L. P., Vilaysane, A., Seamone, M. E., Feng, H., Armstrong, G. D., Tschopp, J., Macdonald, J. A., Muruve, D. A. and Beck, P. L. 2010. *Clostridium difficile* toxin-induced inflammation and intestinal injury are mediated by the inflammasome. Gastroenterology 139(2): 542-52, 552.e1-3.

Nieminen, A., Ramshesh, V. K. and Lemasters, J. J. 2008. Use of Fluorescent Reporters to Measure Mitochondrial Membrane Potential and the Mitochondrial Permeability Transition. In: Dykens, J. A. and Will, Y. (eds.). Drug-induced Mitochondrial Dysfunction. John Wiley & Sons, Hoboken, N.J., USA. p. 413-431.

Nijtmans, L. G. J., Ugalde, C., van den Heuvel, L. P. and Smeitink, J. A. M. 2004. Function and dysfunction of the oxidative phosphorylation system. In: Koehler, C. M. and Bauer, M. F. (eds.). Mitochondrial function and biogenesis. Springer, Berlin, Germany. p. 149-176.

Nilsson, A., Kihlstrom, E., Lagesson, V., Wessen, B., Szponar, B., Larsson, L. and Tagesson, C. 2004. Microorganisms and volatile organic compounds in airborne dust from damp residences. Indoor Air 14(2): 74-82.

Noskin, G. A., Suriano, T., Collins, S., Sesler, S. and Peterson, L. R. 2001. *Paenibacillus macerans* pseudobacteremia resulting from contaminated blood culture bottles in a neonatal intensive care unit. Am. J. Infect. Control 29(2): 126-129.

Novak, E. A. and Mollen, K. P. 2015. Mitochondrial dysfunction in inflammatory bowel disease. Front. Cell. Dev. Biol. 3: 62.

Olano, C., García, I., González, A., Rodriguez, M., Rozas, D., Rubio, J., Sánchez-Hidalgo, M., Braña, A. F., Méndez, C. and Salas, J. A. 2014. Activation and identification of five clusters for secondary metabolites in *Streptomyces albus* J1074. Microb. Biotechnol. 7(3): 242-256.

Ouyang, J., Pei, Z., Lutwick, L., Dalal, S., Yang, L., Cassai, N., Sandhu, K., Hanna, B., Wieczorek, R. L., Bluth, M. and Pincus, M. R. 2008. *Paenibacillus thiaminolyticus*: A New Cause of Human Infection, Inducing Bacteremia in a Patient on Hemodialysis. Ann. Clin. Lab. Sci. 38(4): 393-400.

Paananen, A., Mikkola, R., Sareneva, T., Matikainen, S., Andersson, M., Julkunen, I., Salkinoja-Salonen, M. S. and Timonen, T. 2000. Inhibition of human NK cell function by valinomycin, a toxin from Streptomyces griseus in indoor air. Infect. Immun. 68(1): 165-169.

Pandya, J. D., Nukala, V. N. and Sullivan, P. G. 2013. Concentration dependent effect of calcium on brain mitochondrial bioenergetics and oxidative stress parameters. Front. Neuroenergetics 5: 10.

Park, C. N., Lee, J. M., Lee, D. and Kim, B. S. 2008. Antifungal activity of valinomycin, a peptide antibiotic produced by *Streptomyces* sp. strain M10 antagonistic to *Botrytis cinerea*. J. Microbiol. Biotechnol. 18(5): 880-884.

Patterson, M. F., McKay, A. M., Connolly, M. and Linton, M. 2012. The effect of high hydrostatic pressure on the microbiological quality and safety of carrot juice during refrigerated storage. Food Microbiol. 30(1): 205-212.

Peltola, J., Andersson, M. A., Haahtela, T., Mussalo-Rauhamaa, H., Rainey, F. A., Kroppenstedt, R. M., Samson, R. A. and Salkinoja-Salonen, M. S. 2001a. Toxic-metabolite-producing bacteria and fungus in an indoor environment. Appl. Environ. Microbiol. 67(7): 3269-3274.

Peltola, J. S., Andersson, M. A., Kampfer, P., Auling, G., Kroppenstedt, R. M., Busse, H. J., Salkinoja-Salonen, M. S. and Rainey, F. A. 2001b. Isolation of toxigenic *Nocardiopsis* strains from indoor environments and description of two new *Nocardiopsis* species, *N. exhalans* sp. nov. and *N. umidischolae* sp. nov. Appl. Environ. Microbiol. 67(9): 4293-4304.

Peltola, J., Ritieni, A., Mikkola, R., Grigoriev, P. A., Pocsfalvi, G., Andersson, M. A. and Salkinoja-Salonen, M. S. 2004. Biological effects of *Trichoderma harzianum* peptaibols on mammalian cells. Appl. Environ. Microbiol. 70(8): 4996-5004.

Pereira, C. V., Oliveira, P. J., Will, Y. and Nadanaciva, S. 2012. Mitochondrial bioenergetics and drug-induced toxicity in a panel of mouse embryonic fibroblasts with mitochondrial DNA single nucleotide polymorphisms. Toxicol. Appl. Pharmacol. 264(2): 167-181.

Piipari, R. and Keskinen, H. 2005. Agents causing occupational asthma in Finland in 1986-2002: cow epithelium bypassed by moulds from moisture-damaged buildings. Clin. Exp. Allergy 35(12): 1632-1637.

Pirttijärvi, T. 2000. Ph. D. Thesis. Contaminant Aerobic Sporeforming Bacteria in the Manufacturing Processes of Food Packaging Board and Food. Dissertationes Biocentri Viikki Universitatis Helsingiensis 14/2000: 53 pp. + 4 annexes.

Pirttijärvi, T. S., Graeffe, T. H. and Salkinoja-Salonen, M. S. 1996. Bacterial contaminants in liquid packaging boards: assessment of potential for food spoilage. J. Appl. Bacteriol. 81(4): 445-458.

Pitchayawasin, S., Isobe, M., Kuse, M., Franz, T., Agata, N. and Ohta, M. 2004. Molecular diversity of cereulide detected by means of nano-HPLC-ESI-Q-TOF-MS. Int. J. Mass Spectrom. 235(2): 123-129.

Piuri, M., Sanchez-Rivas, C. and Ruzal, S. M. 1998. A novel antimicrobial activity of a *Paenibacillus polymyxa* strain isolated from regional fermented sausages. Lett. Appl. Microbiol. 27(1): 9-13.

Portnoy, J. M., Kwak, K., Dowling, P., VanOsdol, T. and Barnes, C. 2005. Health effects of indoor fungi. Ann. Allergy Asthma Immunol. 94(3): 313-320.

Priest, F. G. 2009. Genus I: *Paenibacillus*. In: De Vos, P., Garrity, G. M., Jones, D., Krieg, N. R., Ludwig, W., Rainey, F. A., Schleife, K.-H. and Whitman, W. B. (eds.). Bergey's Manual of Systematic Bacteriology, Vol. 3: The *Firmicutes*. Springer, New York, NY, USA. p. 269-296.

Pruimboom-Brees, I., Boucher, G., Jakowski, A. and Wolfgang, J. 2008. Assessment of Mitochondrial Dysfunction by Microscopy. In: Dykens, J. A. and Will, Y. (eds.). Drug-induced Mitochondrial Dysfunction. John Wiley & Sons, Hoboken, N.J., USA. p. 507-538.

Putus, T. 2014. Home ja terveys: kosteusvauriohomeiden, hiivojen ja sädesienten esiintyminen sekä terveyshaitat. Suomen Ympäristö- ja Terveysalan Kustannus, Pori, Finland. 144 pp. [in Finnish]

Qian-Cutrone, J., Huang, S., Shu, Y., Vyas, D., Fairchild, C., Menendez, A., Krampitz, K., Dalterio, R., Klohr, S. and Gao, Q. 2002. Stephacidin A and B: Two structurally novel, selective inhibitors of the testosterone-dependent prostate LNCaP cells. J. Am. Chem. Soc. 124(49): 14556-14557.

Raaska, L., Sillanpää, J., Sjöberg, A. M. and Suihko, M. L. 2002. Potential microbiological hazards in the production of refined paper products for food applications. J. Ind. Microbiol. Biotechnol. 28(4): 225-231.

Rajkovic, A., Uyttendaele, M., Vermeulen, A., Andjelkovic, M., Fitz-James, I., in 't Veld, P., Denon, Q., Vérhe, R. and Debevere, J. 2008. Heat resistance of *Bacillus cereus* emetic toxin, cereulide. Lett. Appl. Microbiol. 46(5): 536-541.

Rajkovic, A., Grootaert, C., Butorac, A., Cucu, T., De Meulenaer, B., van Camp, J., Bracke, M., Uyttendaele, M., Bačun-Družina, V. and Cindrić, M. 2014. Sub-emetic toxicity of *Bacillus cereus* toxin cereulide on cultured human enterocyte-like Caco-2 cells. Toxins (Basel) 6(8): 2270-2290.

Rajkovic, A. 2014. Microbial toxins and low level of foodborne exposure. Trends Food Sci. Technol. 38(2): 149-157.

Ranieri, M. L., Huck, J. R., Sonnen, M., Barbano, D. M. and Boor, K. J. 2009. High temperature, short time pasteurization temperatures inversely affect bacterial numbers during refrigerated storage of pasteurized fluid milk. J. Dairy Sci. 92(10): 4823-4832.

Ranieri, M. L., Ivy, R. A., Mitchell, W. R., Call, E., Masiello, S. N., Wiedmann, M. and Boor, K. J. 2012. Real-time PCR detection of *Paenibacillus* spp. in raw milk to predict shelf life performance of pasteurized fluid milk products. Appl. Environ. Microbiol. 78(16): 5855-5863.

Raulio, M., Jarn, M., Ahola, J., Peltonen, J., Rosenholm, J. B., Tervakangas, S., Kolehmainen, J., Ruokolainen, T., Narko, P. and Salkinoja-Salonen, M. 2008. Microbe repelling coated stainless steel analysed by field emission scanning electron microscopy and physicochemical methods. J. Ind. Microbiol. Biotechnol. 35(7): 751-760.

Reboli, A. C., Bryan, C. S. and Farrar, W. E. 1989. Bacteremia and infection of a hip prosthesis caused by *Bacillus alvei*. J. Clin. Microbiol. 27(6): 1395-1396.

Reed, P. W. and Lardy, H. A. 1972. A23187: a divalent cation ionophore. J. Biol. Chem. 247(21): 6970-6977.

Riclea, R., Aigle, B., Leblond, P., Schoenian, I., Spiteller, D. and Dickschat, J. S. 2012. Volatile lactones from streptomycetes arise via the antimycin biosynthetic pathway. Chembiochem 13(11): 1635-1644.

Rieg, S., Martin Bauer, T., Peyerl-Hoffmann, G., Held, J., Ritter, W., Wagner, D., Kern, W. V. and Serr, A. 2010. *Paenibacillus larvae* bacteremia in injection drug users. Emerg. Infect. Dis. 16(3): 487-489.

Rintala, H., Hyvärinen, A., Paulin, L. and Nevalainen, A. 2004. Detection of streptomycetes in house dust - comparison of culture and PCR methods. Indoor Air 14(2): 112-119.

Rintala, H., Pitkäranta, M., Toivola, M., Paulin, L. and Nevalainen, A. 2008. Diversity and seasonal dynamics of bacterial community in indoor environment. BMC Microbiol. 8: 56-2180-8-56.

Rintala, H., Pitkäranta, M. and Täubel, M. 2012. Microbial Communities Associated with House Dust. Adv. Appl. Microbiol. 78: 75-120.

Rock, K. L., Lai, J. and Kono, H. 2011. Innate and adaptive immune responses to cell death. Immunol. Rev. 243(1): 191-205.

Roig, M., Meca, G., Marin, R., Ferrer, E. and Manes, J. 2014. Antibacterial activity of the emerging *Fusarium* mycotoxins enniatins A, A_1, A_2, B, B_1, and B_4 on probiotic microorganisms. Toxicon 85: 1-4.

Rottenberg, H. 2002. Flow Cytometric Analysis of Mitochondrial Function. In: Lemasters, J. J. and Nieminen, A. (eds.). Mitochondria in Pathogenesis. Kluwer Academic, New York, NY, USA. p. 3-20.

Roux, V. and Raoult, D. 2004. *Paenibacillus massiliensis* sp. nov., *Paenibacillus sanguinis* sp. nov. and *Paenibacillus timonensis* sp. nov., isolated from blood cultures. Int. J. Syst. Evol. Microbiol. 54(Pt 4): 1049-1054.

Rudblad, S., Andersson, K., Stridh, G., Bodin, L. and Juto, J. 2002. Slowly decreasing mucosal hyperreactivity years after working in a school with moisture problems. Indoor Air 12(2): 138-144.

Rudel, T., Kepp, O. and Kozjak-Pavlovic, V. 2010. Interactions between bacterial pathogens and mitochondrial cell death pathways. Nat. Rev. Microbiol. 8(10): 693-705.

Salo, J., Andersson, M. A., Mikkola, R., Kredics, L., Viljanen, M. and Salkinoja-Salonen, M. 2015. Vapor as a carrier of toxicity in a health troubled building. Proceedings of: Healthy Buildings 2015 – Europe (ISIAQ International), Eindhoven, The Netherlands, May 18 -20, 2015, Paper ID526, E.1 Sources & Exposure, Source control. 8 pp.

Sauni, R., Verbeek, J. H., Uitti, J., Jauhiainen, M., Kreiss, K. and Sigsgaard, T. 2015. Remediating buildings damaged by dampness and mould for preventing or reducing respiratory tract symptoms, infections and asthma. Cochrane Database Syst. Rev. 2: D007897.

Scallan, E., Griffin, P. M., Angulo, F. J., Tauxe, R. V. and Hoekstra, R. M. 2011a. Foodborne illness acquired in the United States - unspecified agents. Emerg. Infect. Dis. 17(1): 16-22.

Scallan, E., Hoekstra, R. M., Angulo, F. J., Tauxe, R. V., Widdowson, M. A., Roy, S. L., Jones, J. L. and Griffin, P. M. 2011b. Foodborne illness acquired in the United States--major pathogens. Emerg. Infect. Dis. 17(1): 7-15.

Scheffler, I. E. 2008. Mitochondria (2^{nd} ed.). Wiley-Liss, Hoboken, NJ, USA. 462 pp.

Scheldeman, P., Goossens, K., Rodriguez-Diaz, M., Pil, A., Goris, J., Herman, L., De Vos, P., Logan, N. A. and Heyndrickx, M. 2004. *Paenibacillus lactis* sp. nov., isolated from raw and heat-treated milk. Int. J. Syst. Evol. Microbiol. 54(Pt 3): 885-891.

Schiffer, C., Müller, A., Egeberg, D. L., Alvarez, L., Brenker, C., Rehfeld, A., Frederiksen, H., Wäschle, B., Kaupp, U. B., Balbach, M., Wachten, D., Skakkebaek, N. E., Almstrup, K. and Strünker, T. 2014. Direct action of endocrine disrupting chemicals on human sperm. EMBO Rep. 15(7): 758-765.

Schild, H. A., Fuchs, S. W., Bode, H. B. and Grunewald, B. 2014. Low-molecular-weight metabolites secreted by *Paenibacillus larvae* as potential virulence factors of American foulbrood. Appl. Environ. Microbiol. 80(8): 2484-2492.

Schmidt, V. S., Kaufmann, V., Kulozik, U., Scherer, S. and Wenning, M. 2012. Microbial biodiversity, quality and shelf life of microfiltered and pasteurized extended shelf life (ESL) milk from Germany, Austria and Switzerland. Int. J. Food Microbiol. 154(1-2): 1-9.

Seipke, R. F., Barke, J., Brearley, C., Hill, L., Yu, D. W., Goss, R. J. and Hutchings, M. I. 2011. A single *Streptomyces* symbiont makes multiple antifungals to support the fungus farming ant *Acromyrmex octospinosus*. PLoS One 6(8): e22028.

Seipke, R. F., Kaltenpoth, M. and Hutchings, M. I. 2012. *Streptomyces* as symbionts: an emerging and widespread theme? FEMS Microbiol. Rev. 36(4): 862-876.

Sena, C. M., Pereira, A. M. and Seica, R. 2013. Endothelial dysfunction - a major mediator of diabetic vascular disease. Biochim. Biophys. Acta 1832(12): 2216-2231.

Sener, A. and Malaisse, W. J. 1987. Stimulation by D-glucose of mitochondrial oxidative events in islet cells. Biochem. J. 246(1): 89-95.

Serban, D. E. 2015. Microbiota in Inflammatory Bowel Disease Pathogenesis and Therapy: Is It All About Diet? Nutr. Clin. Pract. 30(6): 760-779.

Shenker, B. J., Ojcius, D. M., Walker, L. P., Zekavat, A., Scuron, M. D. and Boesze-Battaglia, K. 2015. *Aggregatibacter actinomycetemcomitans* cytolethal distending toxin activates the NLRP3 inflammasome in human macrophages, leading to the release of proinflammatory cytokines. Infect. Immun. 83(4): 1487-1496.

Shiota, M., Saitou, K., Mizumoto, H., Matsusaka, M., Agata, N., Nakayama, M., Kage, M., Tatsumi, S., Okamoto, A., Yamaguchi, S., Ohta, M. and Hata, D. 2010. Rapid detoxification of cereulide in *Bacillus cereus* food poisoning. Pediatrics 125(4): e951-5.

Silva, L. J., Crevelin, E. J., Souza, W. R., Moraes, L. A., Melo, I. S. and Zucchi, T. D. 2014. *Streptomyces araujoniae* Produces a Multiantibiotic Complex with Ionophoric Properties to Control *Botrytis cinerea*. Phytopathology 104(12): 1298-1305.

Sivitz, W. I. and Yorek, M. A. 2010. Mitochondrial dysfunction in diabetes: from molecular mechanisms to functional significance and therapeutic opportunities. Antioxid. Redox Signal. 12(4): 537-577.

Sood, S., Steinmetz, H., Beims, H., Mohr, K. I., Stadler, M., Djukic, M., von der Ohe, W., Steinert, M., Daniel, R. and Müller, R. 2014. Paenilarvins: Iturin family lipopeptides from the honey bee pathogen *Paenibacillus larvae*. Chembiochem 15(13): 1947-1955.

Stavru, F. and Cossart, P. 2011. *Listeria* infection modulates mitochondrial dynamics. Commun. Integr. Biol. 4(3): 364-366.

Steglich, W., Fugmann, B. and Lang-Fugmann, S. 2000. Römpp Encyclopedia Natural Products. Georg Thieme Verlag, Stuttgart, Germany. 748 pp.

Stenfors Arnesen, L. P., Fagerlund, A. and Granum, P. E. 2008. From soil to gut: *Bacillus cereus* and its food poisoning toxins. FEMS Microbiol. Rev. 32(4): 579-606.

Stern, N. J., Svetoch, E. A., Eruslanov, B. V., Kovalev, Y. N., Volodina, L. I., Perelygin, V. V., Mitsevich, E. V., Mitsevich, I. P. and Levchuk, V. P. 2005. *Paenibacillus polymyxa* purified bacteriocin to control *Campylobacter jejuni* in chickens. J. Food Prot. 68(7): 1450-1453.

Story, P. and Doube, A. 2004. A case of human poisoning by salinomycin, an agricultural antibiotic. N. Z. Med. J. 117(1190): U799.

Suihko, M. L. and Stackebrandt, E. 2003. Identification of aerobic mesophilic bacilli isolated from board and paper products containing recycled fibres. J. Appl. Microbiol. 94(1): 25-34.

Suominen, I., Suihko, M. L. and Salkinoja-Salonen, M. 1997. Microscopic study of migration of microbes in food-packaging paper and board. J. Ind. Microbiol. Biotechnol. 19(2): 104-113.

Suominen, I., Sproer, C., Kämpfer, P., Rainey, F. A., Lounatmaa, K. and Salkinoja-Salonen, M. 2003. *Paenibacillus stellifer* sp. nov., a cyclodextrin-producing species isolated from paperboard. Int. J. Syst. Evol. Microbiol. 53(Pt 5): 1369-1374.

Sutterwala, F. S., Haasken, S. and Cassel, S. L. 2014. Mechanism of NLRP3 inflammasome activation. Ann. N. Y. Acad. Sci. 1319: 82-95.

Sy-Cordero, A. A., Pearce, C. J. and Oberlies, N. H. 2012. Revisiting the enniatins: a review of their isolation, biosynthesis, structure determination and biological activities. J. Antibiot. (Tokyo) 65(11): 541-549.

Tait, S. W. and Green, D. R. 2012. Mitochondria and cell signalling. J. Cell. Sci. 125(Pt 4): 807-815.

te Giffel, M. C., Wagendorp, A., Herrewegh, A. and Driehuis, F. 2002. Bacterial spores in silage and raw milk. Antonie Van Leeuwenhoek 81(1-4): 625-630.

Tempelaars, M. H., Rodrigues, S. and Abee, T. 2011. Comparative analysis of antimicrobial activities of valinomycin and cereulide, the *Bacillus cereus* emetic toxin. Appl. Environ. Microbiol. 77(8): 2755-2762.

Teng, J. L., Woo, P. C., Leung, K. W., Lau, S. K., Wong, M. K. and Yuen, K. Y. 2003. Pseudobacteraemia in a patient with neutropenic fever caused by a novel *Paenibacillus* species: *Paenibacillus hongkongensis* sp. nov. Mol. Pathol. 56(1): 29-35.

Teplova, V. V., Mikkola, R., Tonshin, A. A., Saris, N. E. and Salkinoja-Salonen, M. S. 2006. The higher toxicity of cereulide relative to valinomycin is due to its higher affinity for potassium at physiological plasma concentration. Toxicol. Appl. Pharmacol. 210(1-2): 39-46.

Teplova, V. V., Tonshin, A. A., Grigoriev, P. A., Saris, N. E. and Salkinoja-Salonen, M. S. 2007. Bafilomycin A1 is a potassium ionophore that impairs mitochondrial functions. J. Bioenerg. Biomembr. 39(4): 321-329.

Thierbach, G. and Reichenbach, H. 1981. Myxothiazol, a new inhibitor of the cytochrome b-c1 segment of the respiratory chain. Biochim. Biophys. Acta 638(2): 282-289.

Thorburn, A. N., Macia, L. and Mackay, C. R. 2014. Diet, metabolites, and "western-lifestyle" inflammatory diseases. Immunity 40(6): 833-842.

Thorsen, L., Budde, B. B., Henrichsen, L., Martinussen, T. and Jakobsen, M. 2009. Cereulide formation by *Bacillus weihenstephanensis* and mesophilic emetic *Bacillus cereus* at temperature abuse depends on pre-incubation conditions. Int. J. Food Microbiol. 134(1-2): 133-139.

Thrasher, J. D. and Crawley, S. 2009. The biocontaminants and complexity of damp indoor spaces: more than what meets the eyes. Toxicol. Ind. Health 25(9-10): 583-615.

Tischer, C. G. and Heinrich, J. 2013. Exposure assessment of residential mould, fungi and microbial components in relation to children's health: Achievements and challenges. Int. J. Hyg. Environ. Health 216(2): 109-114.

Tisoncik, J. R., Korth, M. J., Simmons, C. P., Farrar, J., Martin, T. R. and Katze, M. G. 2012. Into the eye of the cytokine storm. Microbiol. Mol. Biol. Rev. 76(1): 16-32.

Toma, C., Higa, N., Koizumi, Y., Nakasone, N., Ogura, Y., McCoy, A. J., Franchi, L., Uematsu, S., Sagara, J., Taniguchi, S., Tsutsui, H., Akira, S., Tschopp, J., Núñez, G. and Suzuki, T. 2010. Pathogenic *Vibrio* activate NLRP3 inflammasome via cytotoxins and TLR/nucleotide-binding oligomerization domain-mediated NF-kappa B signaling. J. Immunol. 184(9): 5287-5297.

Tonshin, A. A., Teplova, V. V., Andersson, M. A. and Salkinoja-Salonen, M. S. 2010. The *Fusarium* mycotoxins enniatins and beauvericin cause mitochondrial dysfunction by affecting the mitochondrial volume regulation, oxidative phosphorylation and ion homeostasis. Toxicology 276(1): 49-57.

Tuomi, T., Reijula, K., Johnsson, T., Hemminki, K., Hintikka, E., Lindroos, O., Kalso, S., Koukila-Kahkola, P., Mussalo-Rauhamaa, H. and Haahtela, T. 2000. Mycotoxins in crude building materials from water-damaged buildings. Appl. Environ. Microbiol. 66(5): 1899-1904.

Turnbull, P. C., Kramer, J. M., Jorgensen, K., Gilbert, R. J. and Melling, J. 1979. Properties and production characteristics of vomiting, diarrheal, and necrotizing toxins of *Bacillus cereus*. Am. J. Clin. Nutr. 32(1): 219-228.

Tzung, S. P., Kim, K. M., Basanez, G., Giedt, C. D., Simon, J., Zimmerberg, J., Zhang, K. Y. and Hockenbery, D. M. 2001. Antimycin A mimics a cell-death-inducing Bcl-2 homology domain 3. Nat. Cell Biol. 3(2): 183-191.

Täubel, M., Sulyok, M., Vishwanath, V., Bloom, E., Turunen, M., Järvi, K., Kauhanen, E., Krska, R., Hyvärinen, A., Larsson, L. and Nevalainen, A. 2011. Co-occurrence of toxic bacterial and fungal secondary metabolites in moisture-damaged indoor environments. Indoor Air 21(5): 368-375.

van Oss, C., Giese, R. and Docoslis, A. 2005. Hyperhydrophobicity of the water-air interface. J. Dispersion Sci. Technol. 26(5): 585-590.

Vangoitsenhoven, R., Rondas, D., Crevecoeur, I., D'Hertog, W., Baatsen, P., Masini, M., Andjelkovic, M., Van Loco, J., Matthys, C., Mathieu, C., Overbergh, L. and Van der Schueren, B. 2014. Foodborne cereulide causes beta-cell dysfunction and apoptosis. PLoS One 9(8): e104866.

Vangoitsenhoven, R., Maris, M., Overbergh, L., Van Loco, J., Mathieu, C. and Van der Schueren, B. 2015. Cereulide food toxin, beta cell function and diabetes: Facts and hypotheses. Diabetes Res. Clin. Pract. 109(1): 1-5.

Verdier, T., Coutand, M., Bertron, A. and Rogues, C. 2014. A review of indoor microbial growth across building materials and sampling and analysis methods. Build. Environ. 80: 136-149.

Vezina, C., Bolduc, C., Kudelski, A. and Sehgal, S. N. 1976. Antimycin A fermentation. I. Production and selection of strains. J. Antibiot. (Tokyo) 29(3): 248-264.

Vicente-Carrillo, A., Loitto, V., Magnusson, K. E., Rigler, R. and Rodríguez-Martínez, H. 2014. The CatSper receptor family is present in boar spermatozoa. Reprod Dom Anim 49(S3): 99.

Vicente-Carrillo, A., Edebert, I., Garside, H., Cotgreave, I., Rigler, R., Loitto, V., Magnusson, K. E. and Rodríguez-Martínez, H. 2015. Boar spermatozoa successfully predict mitochondrial modes of toxicity: Implications for drug toxicity testing and the 3R principles. Toxicol. in Vitro 29(3): 582-591.

Virtanen, S. M., Roivainen, M., Andersson, M. A., Ylipaasto, P., Hoornstra, D., Mikkola, R. and Salkinoja-Salonen, M. S. 2008. *In vitro* toxicity of cereulide on porcine pancreatic Langerhans islets. Toxicon 51(6): 1029-1037.

Vuorio, R., Andersson, M. A., Rainey, F. A., Kroppenstedt, R. M., Kampfer, P., Busse, H. J., Viljanen, M. and Salkinoja-Salonen, M. 1999. A new rapidly growing mycobacterial species, *Mycobacterium morale* sp. nov., isolated from the indoor walls of a children's day care centre. Int. J. Syst. Bacteriol. 49: 25-35.

Väisänen, O. M., Mentu, J. and Salkinoja-Salonen, M. S. 1991. Bacteria in food packaging paper and board. J. Appl. Bacteriol. 71(2): 130-133.

Walev, I., Reske, K., Palmer, M., Valeva, A. and Bhakdi, S. 1995. Potassium-inhibited processing of IL-1 beta in human monocytes. EMBO J. 14(8): 1607-1614.

Wallace, K. B. and Starkov, A. A. 2000. Mitochondrial targets of drug toxicity. Annu. Rev. Pharmacol. Toxicol. 40: 353-388.

Wang, G. Y. S., Kuramoto, M., Yamada, K., Yazawa, K. and Uemura, D. 1995. Homocereulide, an Extremely Potent Cytotoxic Depsipeptide from the Marine Bacterium *Bacillus cereus*. Chem. Lett. (9): 791-792.

Wojtczak, L. and Zablocki, K. 2008. Basic Mitochondrial Physiology in Cell Viability and Death. In: Dykens, J. A. and Will, Y. (eds.). Drug-induced Mitochondrial Dysfunction. John Wiley & Sons, Hoboken, N.J., USA. p. 3-35.

Wolff, C. H. 2011. Innate immunity and the pathogenicity of inhaled microbial particles. Int. J. Biol. Sci. 7(3): 261-268.

Wong, D. T., Berg, D. H., Hamill, R. H. and Wilkinson, J. R. 1977. Ionophorous properties of narasin, a new polyether monocarboxylic acid antibiotic, in rat liver mitochondria. Biochem. Pharmacol. 26(15): 1373-1376.

Wulff, J. E., Herzon, S. B., Siegrist, R. and Myers, A. G. 2007. Evidence for the rapid conversion of stephacidin B into the electrophilic monomer avrainvillamide in cell culture. J. Am. Chem. Soc. 129(16): 4898-4899.

Yan, L. L., Han, N. N., Zhang, Y. Q., Yu, L. Y., Chen, J., Wei, Y. Z., Li, Q. P., Tao, L., Zheng, G. H., Yang, S. E., Jiang, C. X., Zhang, X. D., Huang, Q., Habdin, X., Hu, Q. B., Li, Z., Liu, S. W., Zhang, Z. Z., He, Q. Y., Si, S. Y. and Sun, C. H. 2010. Antimycin A18 produced by an endophytic *Streptomyces albidoflavus* isolated from a mangrove plant. J. Antibiot. (Tokyo) 63(5): 259-261.

Yang, S., Kim, S., Ryu, J. H. and Kim, H. 2013. Inhibitory activity of *Paenibacillus polymyxa* on the biofilm formation of *Cronobacter* spp. on stainless steel surfaces. J. Food Sci. 78(7): M1036-40.

You, B. R. and Park, W. H. 2010. The effects of antimycin A on endothelial cells in cell death, reactive oxygen species and GSH levels. Toxicol. in Vitro 24(4): 1111-1118.

Zeigler, D. R. 2013. The Family Paenibacillaceae - Bacillus Genetic Stock Center Catalog of Strains Part 5. The Bacillus Genetic Stock Center, Columbus, OH, USA. 28 pp.

Zhao, L., Yang, X., Li, X., Mu, W. and Liu, F. 2011. Antifungal, Insecticidal and Herbicidal Properties of Volatile Components from *Paenibacillus polymyxa* Strain BMP-11. Agric Sci China 10(5): 728-736.

Zhou, R., Yazdi, A. S., Menu, P. and Tschopp, J. 2011. A role for mitochondria in NLRP3 inflammasome activation. Nature 469(7329): 221-225.